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A General Classification of Available Small Seeded Legumes as Hosts for Three Aphids of the "Yellow Clover Aphid Complex"[1,2]

DON C. PETERS and REGINALD H. PAINTER,[3] *Kansas State College, Manhattan*

The spotted alfalfa aphid, the sweetclover aphid, and the yellow clover aphid (fig. 1),[4] constitute a group of closely related aphids that feed on allied genera of legumes. According to Dickson *et al.* (1955), the spotted alfalfa aphid has probably been in the United States since about February 1954. The sweetclover aphid was described in Europe by Börner (1949). The presence of this aphid in the United States seems to have been discovered in connection with surveys to determine the distribution of the spotted alfalfa aphid. The yellow clover aphid was described from Washington, D. C., and is generally present from Maine to Florida where red clover is grown. It has been observed in this country since 1880, according to Davis (1914).

Some of the plant hosts of these aphid species have been reported by several different authors. Davis (1914) reported that it was possible to rear the yellow clover aphids on "*Trifolium pratense*, *T. repens*, alsike, English and Mammoth clover (*T. medium*)." He reported that the results on *Medicago hispida* were inconclusive, and that the yellow clover aphid would not survive on alfalfa. Börner (1949), in describing the sweetclover aphid, listed *Melilotus alba* and *M. officinalis* as host plants. Dickson & Reynolds (1955) reported that, in addition to alfalfa, the spotted alfalfa aphid was found on *Medicago hispida*, *Medicago lupulina*, *Melilotus indica*, and *Trifolium alexandrinum*. They also reported that red clover, "ladino," "trefoil" and vetch are not suitable hosts. Dickson *et al.* (1955) listed the following additional hosts of the spotted alfalfa aphid: *Medicago falcata*, *Trifolium fragiferum*, *Trifolium incarnatum*, *T. hybridum*, and *Melilotus officinalis*. All of the above authors fail to identify the strain of the plant species studied with a record number; therefore, accurate confirmation is not possible.

MATERIALS AND METHODS.—The diversity in the climatic requirements of many of the legumes studied made it necessary to do most of the work in the greenhouse. Some field observations were made on the common native and cultivated legumes. Field observations were made by counting the number of aphids collected in a 15-inch beating net that had been dyed black to contrast with the yellow-green color of the aphids.

The first step in the greenhouse work was to obtain cultures of the three species of aphids. These cultures usually were brought in on plants from infested fields. The spotted alfalfa aphid culture was a composite of collections from seven counties in different regions of Kansas. The yellow clover aphid culture was a composite from collections in three counties in the red clover growing area of northeastern Kansas. Collections from within two counties were mixed and used in the sweetclover aphid culture. All the aphids were reared on greenhouse-grown plants of their respective hosts. The host varieties generally used were Buffalo or Kansas Common alfalfa, Midland red clover, and Madrid or biennial white sweetclover.

The plants for evaluating host range were grown individually in 4-inch clay pots. The soil used was a mixture of 6 parts black loam, 1 part sheep manure, and 1 part sand. The plants were generally 6 weeks to 2 months old when the tests were made and most of them had not begun to flower. Tests were conducted at temperatures of about 75 degrees F.

It had been intended to cage the individual plants in cylinders of cellulose acetate for the host relationship studies, but preliminary tests indicated that the acetate material on hand was phytotoxic. As an alternate method, the pots were placed in large cages (27″×24″×18″) covered with 32-mesh per inch Lumite. Each cage held 20 pots. The plants were infested by shaking 10 aphids of one of the respective species on each plant. Three plants per species or strain were included in the first tests. Counts of the number of aphids per plant were taken on the seventh and fourteenth day after infestation. Nontoxic caging material was obtained in time to be used for caging individual plants on about the last half of the plant species tested.

The counts in the large cages did not indicate entirely the reproductive capacity of the 10 aphids placed on the

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[3] Graduate Research Assistant and Entomologist, respectively. Thanks are due Max M. Hoover, Edwin James, H. O. Graumann, E. A. Hollowell, E. L. Sorensen, and others of the U. S. Department of Agriculture, W. L. Richardson of the Department of Agronomy, Oklahoma Agricultural and Mechanical College, and K. L. Anderson and C. E. Wassom of the Department of Agronomy, Kansas State College, for supplying seed, and R. G. Dahms, Entomology Research Branch Agric. Research Service, U. S. D. A., for original proposals in this study.

[4] *Pterocallidium sp.*, *Myzocallidium riehmi* Börner, and *Pterocallidium trifolii* (Monell) are the respective scientific names currently being used by workers of the U. S. Department of Agriculture.

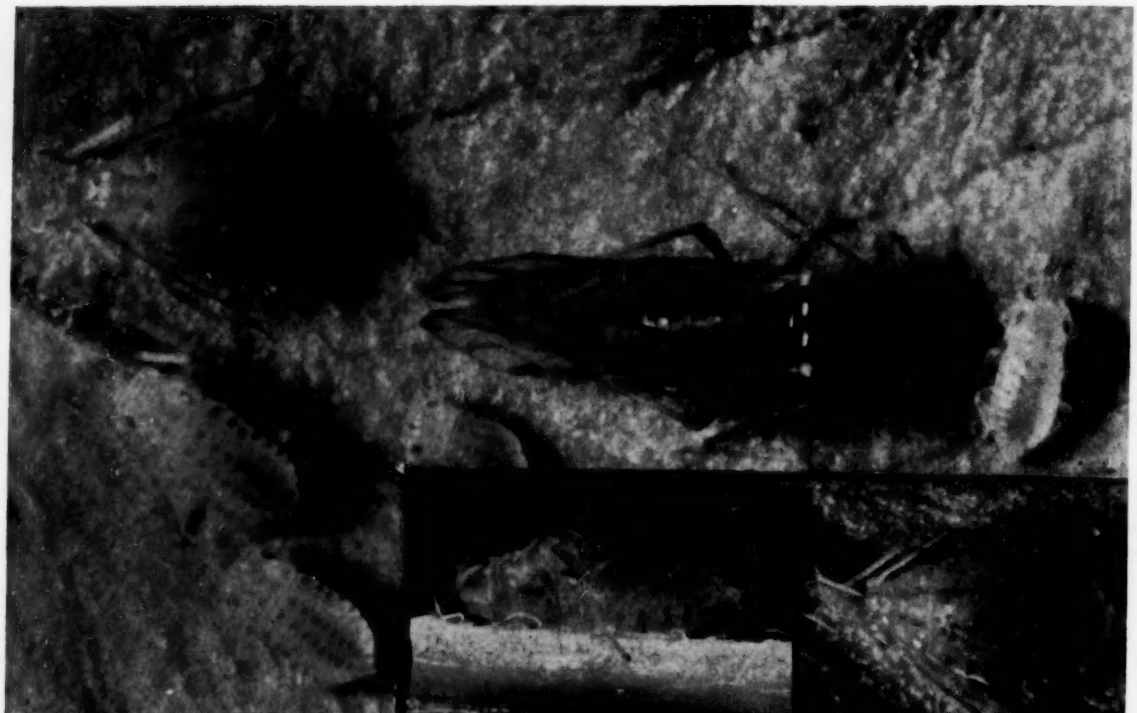

FIG. 1.—Adults of the "Yellow Clover Aphid Complex." Lower right, yellow clover aphid; lower center, spotted alfalfa aphid; others, sweetclover aphid.

plant, since it was possible for some of the aphids to move from non-preferred host plants to preferred ones in the same cage. Where results from the first test suggested that the aphids could not survive on a particular plant species, five or more plants of that species were caged individually in cellulose nitrate cylinders with nylon tops and windows, and each plant infested with 50 or more aphids. Counts of the number of aphids present were made on the third and seventh day after infestation and, if none of the aphids or progeny survived after 7 days on any of the host plants of the species, that species was classified as "apparently immune" (I) to the aphid concerned. The spotted alfalfa aphid was able to live for periods longer than 7 days on some plants of *Trifolium fragiferum* and *Lespedeza stipulacea*. This relationship has been given the classification of subsistence or "possible host" (PH). Plants on which the aphids were able to survive and reproduce were classified as hosts with three arbitrary degrees of susceptibility recognized.

If the number of aphids from an original infestation of 10 per plant did not exceed 20 on any of the plants of a species at the end of the 2-week period, the species was classified as "resistant to semi-susceptible" (S). When the number of aphids at the end of the period was between 20 and 100 and the plants were not wilted or dead as a result of aphid feeding, the species was classified as "susceptible" (SS). In the event that any of the plants were killed by the aphids or the number of aphids at the end of 2 weeks was over 100, the species was classified as "highly susceptible" (SSS) to that aphid species. Each species was given the *classification of the most susceptible plant* in the sample. Plant species studied in the field were classified as hosts or non-hosts.

More than 70 species of plants were observed or tested either in the field or greenhouse, in the course of these observations. Further studies now in progress may change the ratings of some species, but it is believed such changes will be due to refinements in classification.

RESULTS.—Field observations, during the fall of 1955, consisting of sweepings and beatings of one or more species or varieties of the following legume genera failed to produce any aphids of the yellow clover aphid complex: *Astragalus, Bonjeanid, Coronilla, Desmanthus, Hedysarum, Lathyrus, Lespedeza, Lotus, Onobrychis, Ornithopus, Schrankia,* and *Vica*. Aphids of all three species studied were collected from their respective hosts in the same nursery.

The spotted alfalfa aphid was observed on the following species of *Medicago: sativa, falcata, lupulina,* and *ruthenica*. The yellow clover aphid was collected from red clover (*Trifolium pratense*) and zigzag clover (T. *medium*), but was not found on *T. ambiguum, T.hybridum,* or *T. repens* (Ladino). Sweetclover aphids were collected from *Melilotus alba, M. officinalis,* and *Melilotus* spp. (annual sweetclover).

Results from the greenhouse work are summarized in table 1. In general, the members of the yellow clover aphid complex in America find most of their hosts in four genera: *Medicago, Melilotus, Trifolium,* and *Trigonella*. *Dalea alopecuroides* and *Lespedeza stipulacea* possibly are additional hosts.

The spotted alfalfa aphid was able to feed and repro-

Table 1.—A classification[a] of small seeded legumes as hosts of the spotted alfalfa aphid, yellow clover aphid and sweetclover aphid, under greenhouse conditions.

Species	Record No.	Common Name	Classification as Host of		
			Spotted Alfalfa Aphid	Yellow Clover Aphid	Sweetclover Aphid
Alysicarpus sp.[b]	FC 189493	Alyce clover	I	I	I
Coronilla varia		Crown vetch	I	I	I
Dalea alopecuroides	FC 231728	Foxtail dalea	I	Sv	I
Desmanthus illinoensis		Illinois bundleflower	I	I	I
Desmodium illinoense		Illinois tickclover	I	I	I
Lespedeza					
cuneata	KL 365-50	Sericia lespedeza	I	I	I
daurica var. schmiadae	KL 4-50		I	I	I
hedysarioides	KL 238-50		I	I	I
stipulacea	FC 32635	Korean lespedeza	PHv	Phv	I
striata	FC 32636	Common lespedeza	I	I	I
Lotus					
corniculatus	KL 268-49	Birdsfoot trefoil	I	I	I
uliginosis[c]		Big trefoil	I	I	I
Medicago					
arabica	PI 206693	Spotted burclover	Sv	I	I
arborea	PI 197827	Tree medic	SSS	S	I
blancheana	PI 197333		S	I	I
ciliaris	PI 197525		SSS	SS	I
falcata	KL 347		SSSv	I	I
hispida	FC 24598	California burclover	SSS	S	I
lupulina	PI 189128	Black medic	SSSv	SS	I
lupulina	FC 24600		SSSv	SS	I
lupulina	PI 197743		Sv	Sv	I
orbicularis	PI 170533	Button medic	SSS	I	I
praecox	PI 212948		SSS	SSS	I
rigidula	PI 217647	Tifton medic	SSS	I	I
sativa	FC 32984	Buffalo alfalfa	SSSv	I	I
sativa	FC 33087[d]	Lahontan alfalfa	SSv	I	I
scutellata	FC 189571	Snail medic	Sv	I	I
sp.	PI 204892		SS	SS	I
truncaiata	PI 190083		Sv	I	I
tuberculata	PI 197375	Cogwheel medic	Sv	I	I
Melilotus					
alba	PI 193292	Biennial white sweetclover	Sv	I	Sv
alba	Kans. sel.		Sv	I	SSSv
dentata	PI 205531 A-53		SS	Sv	SSS
hirsuta	PI 129697		Sv	I	SSS
indica	FC 24187	Annual yellow sweetclover (sour clover.)	SSS	SS	SSS
italica	PI 202510		SSS	SSS	SSS
leucantha	A# 3977		I	I	SSS
messanensis	PI 198966		S	S	SSSv
officinalis	FC 32599	Yellow sweetclover	Sv	I	SSSv
ruthenica	A# 1295		I	I	SSS
segetalis	PI 202512		SSS	SSS	SSS
sp.	PI 90755	Annual sweetclover	Sv	I	SSS
sp.	FC 32577	Annual sweetclover	Sv	I	SSSv
suaveolens		Daghestan sweetclover	Sv	I	SSS
taurica	PI 67512		Sv	I	SS
Pueraria thunbergiana	FC 189730	Kudzu	I	I	I
Trifolium					
agrarium	PI 1714814-B-52	Hop clover	SS	SS	I
alexandrinum	FC 24479	Berseem	SS	SS	I
arvense	FC 204518	Rabbitfoot clover	I	SSS	I
campestre	FC 207939	Large hop clover	I	SSS	I
dubium	PI 222131	Small hop clover	SS	SSS	I

[a] See text for comments on classification. I—immune. PH—possible host. S—resistant to semisusceptible. SS—susceptible. SSS—highly susceptible. v—These strains show evidence of individual plants that differ appreciably from most of the population in susceptibility or resistance to the aphid concerned.

[b] Probably *A. vaginalis*.

[c] Rank of this species based on inability of aphids to infest 20 to 30 plants placed in aphid culturing cages.

[d] Results with a larger sample of Lahontan seedlings indicated that approximately 20% of the plants may die after heavy spotted alfalfa aphid infestation.

(*Continued on next page*)

Table 1—(continued)

Species	Record No.	Common Name	Classification as Host of: Spotted Alfalfa Aphid	Yellow Clover Aphid	Sweetclover Aphid
fragiferum	FC 24868	Strawberry clover	Phv	SS	I
glomeratum	FC 201211	Cluster clover	I	SSS	I
hirtum	FC 206761	Rose clover	I	Sv	I
hybridum (2n)	FC 24296	Alsike clover	S	SS	I
hybridum (4n)	PI 194148	Alsike clover	S	SS	I
incarnatum	FC 32967	Crimson clover	SS	SS	I
lappaceum	PI 170822	Lappa clover	I	S	I
medium	FC 206485	Zigzag clover	I	SSS	I
medium	WDL 66B-1954	Zigzag clover	I	SSS	I
nigrescens	FC 210354	Ball clover	I	SS	I
pratense	FC 187224	Red clover	I	SSS	I
pratense	FC 32619	Wisc. Syn. red clover	I	SSSv	I
procumbens	FC 32228	Low hop clover	S	SSS	I
radiosum	FC 206771		I	SSS	I
repens	PI 166371	White clover	I	Sv	I
resupinatum	PI 163314	Persian clover	I	Sv	I
subterraneum	PI 201215	Sub clover	I	SS	I
xerocaphulum	PI 173818		SSv	SSS	I
Trigonella					
balansae	PI 164566		SSS	SSS	SS
coerulea	PI 206901	Bluewhite trigonella	SSSv	SS	SSS
foenum graecium	PI 204527	Fenugreek	Sv	I	Sv
kotchy[e]	PI 206775		SS	SS	SS

[e] Records based on only one to three plants which were all that germinated.

duce on some plants of all of the *Medicago* species studied. Certain plants or strains of *M. sativa*, *M. falcata*, *M. lupulina*, and others indicated as variable in table 1 showed a considerable degree of resistance to the spotted alfalfa aphid. All *Melilotus* species studied had some plants on which the spotted alfalfa aphid could feed, but with the exception of *M. indica*, *M. italica*, *M. segetalis*, and *M. dentata*, which were quite susceptible, the other *Melilotus* species were composed mostly of plants that were highly resistant or immune to the spotted alfalfa aphid. Eight of the 20 *Trifolium* species studied were susceptible in varying degrees to the spotted alfalfa aphid but none was highly susceptible. The four species of *Trigonella* were all susceptible to the spotted alfalfa aphid but in varying degrees.

Some plants of all of the *Trifolium* species studied served as hosts for the yellow clover aphid, but most plants of *T. repens*, *T. hirtum*, *T. resupinatum* and *T. fragiferum* were immune. Almost all the plants of *T. pratense* studied seemed to be uniformly susceptible to the yellow clover aphid, but examination of more plants of several varieties including Wisconsin Synthetic, showed the presence of resistant plants especially in the latter variety. Of the 15 *Medicago* species studied, 6 were susceptible to some extent to the yellow clover aphid. Plants of *M. ciliaris*, *M. lupulina*, *M. praecox*, and *Melilotus* sp. (P. I. 204892) were susceptible but only those of *M. praecox* were highly susceptible. Some plants of *Dalea alopecuroides* were susceptible to the yellow clover aphid.

The sweetclover aphid was found to have as hosts only species of *Melilotus* and *Trigonella*. Most of the species of *Melilotus* were highly susceptible, but occasional plants had various degrees of resistance. Some plants of *M. alba*, P. I. 193292, were apparently immune to sweetclover aphids, and aphid reproduction was retarded on most other plants of this strain. *M. taurica* and *M. hirsuta* were slightly less susceptible than the others. The four *Trigonella* species gave a reaction to the sweetclover aphid that was essentially similar to the reaction of the spotted alfalfa aphid on the same plant species.

Melilotus indica, *M. italica*, *M. segetalis*, *Trigonella balansae*, *T. coerulea* and *T. kotchy* were the only host plants found to be generally susceptible to all three species of aphids.

Direct and gradual transfers of aphids from red clover, alfalfa, or sweetclover to each of the other of these hosts were attempted. Direct transfers were made by caging separately 5 to 20 aphids per plant on individual plants of each species. Such tests were made with each of the three aphid species. Gradual transfers were attempted by planting rows of the host species and the other two species in the same flat and allowing the aphids to feed and reproduce at random. Observations on these experiments consisted of weekly or bi-weekly records until the host species was killed by the aphids. With the exception of a few sweetclover plants that were susceptible to the spotted alfalfa aphid, all such attempts to force aphids to feed on the other two economic plant species were fruitless.

Discussion.—It should be kept in mind that this work was done with young and generally non-flowering plants, and that older or mature plants might show a different relationship to the aphids. However, observations on a number of older plants of several species indicated a similar reaction in older and younger plants. Sexual forms of the yellow clover aphid and the sweetclover aphid were common during a portion of this study, and it is

possible that some of the plant species are more susceptible to these two aphid species than table 1 indicates. This difference, if it exists, would be one of degree only since sexual forms were observed to prefer and reproduce upon the same hosts as their parthenogenic precursor relatives.

Variability in host species reactions has been observed in all of the susceptible legume species studied intensively. The size of aphids as measured by weight of alate adults of spotted alfalfa aphids and yellow clover aphids seems to be significantly different on some of the host species and even among plants within a host species. The fecundity studies now in progress also appear to indicate similar variabilities. Each plant species except alfalfa was represented in these studies by three to about 100 individual plants. Intensive search in some host species, particularly the ones designated as variable, or studies under different environmental conditions may reveal a wider range of reactions than that recorded here. This has been repeatedly demonstrated in the search for insect-resistant crop plants. It was illustrated in the study by the difference in host reaction of plants or strains of the following species: *Medicago lupulina*, *M. sativa*, *Melilotus alba*, and *Trifolium pratense*.

Each aphid species has a distinct pattern of host plant species. The sweetclover aphid has the smallest host range mostly limited to *Melilotus*, but within this range most species were highly susceptible. The other two aphid species studied had about the same number of host species but differed in that the spotted alfalfa aphid found more of its hosts within the genus *Medicago* whereas the yellow clover aphid found more hosts within *Trifolium*. There was considerable overlapping of host species for the spotted alfalfa aphid and the yellow clover aphid. Five species of *Medicago* and *Trifolium* were given the same classification to both aphid species. As stated previously nearly all host plant species of the three aphid species were from the four genera: *Medicago*, *Melilotus*, *Trifolium* and *Trigonella*, but of the 53 species in these genera studied, 12 were immune or resistant to all three aphid species. The similarity in reactions of the four *Trigonella* species to each of the aphid species is striking when compared to the wide range of reactions within the other genera.

It is fortunate that none of the three important economic crops in Kansas is attacked by more than one of these three insects. The spotted alfalfa aphid is able to feed and maintain small populations on some plants of most species and varieties of sweetclover studied, so it would be well to test all new sweetclover varieties before releasing them to farmers, to prevent release of varieties composed mainly of plants susceptible to the spotted alfalfa aphid.

Another factor with possible economic implications is the susceptibility of *Dalea alopecuroides* to the yellow clover aphid. While this is the only native legume studied that was found susceptible to any of the aphid species, it emphasizes the need for a more thorough search and study of the native legumes, for such wild hosts could have a definite bearing on insect control practices.

The evolution of host plant relationships of insects has been studied to some extent but not so extensively as its importance warrants. This study of the yellow clover aphid complex is an example of the type of basic biological information needed. The interrelations between these aphid species, plus those in Europe and elsewhere also, and their hosts might, if properly evaluated, shed much light on the evolution of both insect and plant species involved.

Detailed records and counts of aphid progeny produced on the several plant hosts are on file at the Department of Entomology, Kansas State College, Manhattan.

SUMMARY.—The interrelationships between more than 70 small seeded legumes with the spotted alfalfa aphid [currently known as *Therioaphis maculata* (Buckton) or *Pterocallidum* sp.], yellow clover aphid [*Pterocallidum trifolii* (Monell)], and the sweetclover aphid (*Myzocallidium riehmi* Börner) have been studied in cooperation with the Entomology Research Branch, Agricultural Research Service, U. S. Dept of Agriculture. Studies were confined largely to the greenhouse. The sweetclover aphid has its hosts in the two genera, *Melilotus* and *Trigonella;* the other two species have differing host relationships throughout *Medicago*, *Melilotus*, *Trifolium*, and *Trigonella*. Twelve species in these genera were immune to resistant to all three aphids.

Of the legume species studied about 15 were susceptible to the sweetclover aphid while twice that number were susceptible to both of the other two aphid species. Plants of other genera studied were generally immune to all three aphid species. Alfalfa is not attacked by yellow clover aphid or the sweetclover aphid, and most plants of sweetclover are not injured by yellow clover or spotted alfalfa aphids. Six plant species in *Melilotus* and *Trigonella* serve about equally as well as hosts for all three aphid species.

Some plants of *Dalea alopecuroides* were susceptible to the yellow clover aphid. Within most of the plant species studied individual plants differing in reaction to one or more of the aphids species were evident.

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Control of Insect and Mite Pests of Beans in Hawaii[1]

MARTIN SHERMAN and MINORU TAMASHIRO,[2] *University of Hawaii, Agricultural Experiment Station, Honolulu, T. H*

The green bean is subject to attack by a number of serious pests in Hawaii. Sherman *et al.* (1954) reported on the control of several of these pests including the greenhouse whitefly, *Trialeurodes vaporariorum* (Westw.), and the Southern garden leafhopper, *Empoasca solana* De Long. During 1955 additional studies were made on the control of these insects as well as the onion thrips, *Thrips tabaci* Lind., and two species of mites—the two-spotted spider mite, *Tetranychus telarius* (L.), and the broad mite, *Hemitarsonemus latus* (Banks). In all of these tests, the Wade variety of bush bean was used.

PROCEDURE.—In Experiment 1, each treatment-plot consisted of a single row 10 feet long. The rows were spaced 8 feet apart and there was a 5-foot unplanted space between the plots along each row. Each plot contained one hill per foot of row with two plants in each hill. The experimental plots were arranged in a 7 by 7 Latin square.

In Experiments 2 and 3, each plot consisted of a group of three rows 10 feet long. The rows were spaced 4 feet apart and there was a 5-foot unplanted space between the plots along the rows. Adjacent to each three-row plot, an 8-foot space was left unplanted to reduce spray contamination due to drift. Each plot contained one hill per foot of row with two plants in each hill. The experimental plots were arranged in randomized blocks.

The criteria used in the determination of infestation differed with the species involved. In the case of the greenhouse whitefly and the bean leafhopper, infestation was based on the nymph population. Infestation by the onion thrips and the broad mite was determined by the presence of both nymphs and adults. The infestation of the two-spotted spider mite was determined by the adult population present.

Starting with the third hill in each plot-row in Experiment 1 and the third hill in the center row of each plot in Experiments 2 and 3, a total of 10 terminal leaflets of approximately the same age was taken at random from each plot. The entire leaflet was examined for the presence of the bean leafhopper and the onion thrips. However, since it was impractical to count all of the greenhouse whitefly nymphs, two-spotted spider mite adults, or broad mites on each leaflet, a subsampling procedure was used. Four circular impressions were made at random on each leaflet with a No. 10 cork borer. The total area per leaflet encompassed by these impressions was 1018 mm.[2] This area on each leaf was examined and the number of individuals tallied.

RESULTS.—*Experiment 1.*—Wettable powder formulations of the following insecticides were used: heptachlor, Strobane, EPN, endrin, and isodrin. The concentrations at which these materials were applied on May 10, 1955, by power sprayer and the results of the treatments are given in table 1.

The greenhouse whitefly was best controlled by EPN at 2 pounds of formulation per 100 gallons. The use of Strobane and isodrin at 2 and 1 pounds, respectively, significantly reduced the population when compared with the untreated plots 15 days after treatment. Heptachlor at 2 pounds, Strobane at 1 pound and endrin at $2\frac{1}{2}$ pounds per 100 gallons did not appear to control this insect. The results obtained with heptachlor and EPN confirm those obtained earlier (Sherman *et al.* 1954).

The two-spotted spider mite was late in becoming established; however, by May 25, the population in the check plots had increased considerably. Good control of this mite was obtained with the use of EPN, endrin, or isodrin. Heptachlor and Strobane did not appear to control this species.

All of the materials tested gave good control of both the southern garden leafhopper and the onion thrips.

Experiment 2.—Ovex, Aramite, and malathion were each applied as a wettable powder at two concentrations in an attempt to control the broad mite. Each treatment was replicated three times. The concentrations at which these materials were applied by power sprayer on July 13 and July 28, 1955, and the results of the treatments are given in table 2.

All of the treatments resulted in excellent control of the mite 1 week after the initial application, but within 2 weeks the mite population had increased to such an extent that a second application was necessary. Although all of the treatments reduced the infestation 3 days later, only the plants treated with ovex at either 1 or $\frac{1}{2}$ pound of the wettable powder per 100 gallons had a significantly lower population of mites on August 8. Malathion wettable powder was inferior to the other materials.

Experiment 3.—Six treatments to control the broad mite were applied and replicated four times. Malathion, demeton, and schradan were applied as emulsifiable concentrates, while sulfur was applied as a wettable powder. The concentrations at which these materials were applied by power sprayer and the results are given in table 2. A week after the initial application of July 13, 1955, the mite population had been significantly reduced in all of the treated plots. By July 26, however, the population had begun to increase in the plots treated with malathion at 1 pound of actual material per 100 gallons and in the plots treated with either concentration of demeton. Therefore, a second application of these three treatments was made on July 28, 1955.

It appears that under Hawaiian conditions sulfur is superior to the other materials tested for broad mite control. One application of 95% sulfur at 5 pounds of the wettable powder per 100 gallons resulted in practically mite-free plants for a period of 4 weeks. A single application of the malathion emulsifiable concentrate at 2 pounds of actual material per 100 gallons also gave good protection, as did two applications of demeton at 1

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Table 1.—Control of insects and mites attacking green beans in Hawaii.

Insecticide[a] (Per Cent Wettable Powder)		Pounds per 100 Gals.	Greenhouse Whitefly		Two-Spotted Spider Mite		Bean Leafhopper		Onion Thrips[b]	
			Average Number of Nymphs per Leaf Sample (1018 mm.²)		Average Number of Adults per Leaf Sample (1018 mm.²)		Average Number of Nymphs per 10 Leaflets		Average Number of Adults and Nymphs per 10 Leaflets	
			May 9	May 25	May 19	May 25	May 9	May 25	May 9	May 25
Heptachlor	25	2	128.3	43.9	0.4	3.3	10	4.2[d]	26	0.4[d]
Strobane	50	2	126.5	15.1[c]	0.2[c]	6.4	8	0.7[d]	26	0.6[d]
		1	55.4	25.2	0.7	4.1	13	3.2[d]	21	0.4[d]
EPN	25	2	84.9	3.0[d]	0.1[c]	0.3[d]	18	0.1[d]	24	0.7[d]
Endrin	10	2½	90.9	28.9	0.3	1.0[c]	14	0.6[d]	39	0.3[d]
Isodrin	25	1	119.4	17.4[c]	0.6	1.7[c]	10	0.4[d]	30	0.4[d]
Untreated		—	86.0	87.0	1.5	10.5	14	20.6	29	15.1

[a] Date of Application: May 10, 1955. Pre-spray count made May 9.
[b] *Thrips tabaci* Lind. was the most abundant species; *Leucothrips pierci* (Morgan), a common incidental pest on vegetable crops, was also present.
[c] Significant at 5% level.
[d] Significant at 1% level.

pound of actual per 100 gallons. Schradan at 1 pound of active ingredient per 100 gallons gave good protection for a 3-week period.

Table 2.—Control of broad mite, *Hemitarsonemus latus* (Banks), attacking green beans in Hawaii.

Insecticide	Amount per 100 Gal.	Average Number of Broad Mites per Leaf Sample (1018 Mm²)			
		July 19	July 26	Aug. 1	Aug. 8
	Experiment 1				
Ovex 50% W.P.	1 lb.[a]	4.9	11.7	4.4	3.3
	0.5 lb.[a]	3.7	5.3	7.4	10.8
Aramite 15% W.P.	2 lbs.[a]	3.1	11.5	8.3	20.6
	1 lb.[a]	3.7	8.6	9.8	58.9
Malathion 25% W.P.	2 lbs.[a]	4.9	20.7	18.3	106.1
	1 lb.[a]	4.4	23.6	27.6	156.9
Untreated	—	18.4	30.8	123.8	91.7
L.S.D. at 5% level		6.8	none	34.1	71.7
at 1% level		9.4		47.6	—
	Experiment 2				
Sulfur 95% W.P.	5 lbs.[b]	0.1	0.1	0.2	0.5
Malathion 55% E	1–250[b]	4.1	4.7	21.3	30.0
(5 lbs. act./G.)	1–500[a]	5.3	26.7	19.4	60.8
Demeton E	1–200[a]	2.0	12.0	6.2	27.2
(2 lbs. act./G.)	1–400[a]	3.8	21.6	13.5	57.6
Schradan E	1–400[b]	1.8	4.7	28.3	57.3
(4 lbs. act./G.)					
Untreated	—	24.8	41.8	125.7	107.6
L.S.D. at 5% level		9.2	20.2	17.2	53.1
at 1% level		12.5	27.5	23.8	72.7

[a] Dates of application: July 13 and 28, 1955.
[b] Date of application: July 13, 1955.

SUMMARY.—Small plot experiments were conducted in 1955 to determine the efficacy of insecticide and acaricide sprays in controlling several pests of green beans.

EPN wettable powder at 0.063% gave excellent control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westw.), while Strobane at 0.13% and isodrin at 0.03% gave good control.

EPN wettable powder at 0.063% and endrin and isodrin at 0.03% gave good control of the two-spotted spider mite, *Tetranychus telarius* (L.).

All of the materials tested controlled the southern garden leafhopper, *Empoasca solana* De Long, and the onion thrips, *Thrips tabaci* Lind.

Sulfur appeared to be superior to the other materials tested against the broad mite, *Hemitarsonemus latus* (Banks), although the use of ovex wettable powder at 0.03%, malathion emulsifiable concentrate at 0.25%, and demeton emulsifiable concentrate at 0.125% resulted in good control. Schradan emulsifiable concentrate at 0.125% gave only fair control.

REFERENCE CITED

Sherman, Martin, Minoru Tamashiro, and E. T. Fukunaga. 1954. Control of greenhouse whitefly and other insects on beans in Hawaii. Jour. Econ. Ent. **47**(3): 530–5.

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The Prolonged Maintenance of the Western Subterranean Termite in the Laboratory with Moisture Gradient Tubes

ROY J. PENCE, *Department of Entomology, University of California, Los Angeles*

It is very difficult to maintain the western subterranean termite, *Reticulitermes hesperus* Banks, in the laboratory for prolonged and detailed observation of its behavior and feeding habits on treated and untreated wood.

The subterranean termite is extremely sensitive to any environment not in keeping with the restricted optimum microclimate it requires for normal existence. Excessive moisture or dryness will result in high mortality and regulated conditions are difficult to maintain when changing outside influences tend to upset the balance. Too often the check lots in experiments conducted in the laboratory have suffered a mortality equal to that resulting from treated wood. This prompted the development of a simple technique for keeping subterranean termites in the laboratory by duplicating as closely as possible the field conditions to which they are accustomed.

Field observations have shown that subterranean termites will often extend tubes many feet upward and away from the moist earth they require. It is known that termites will feed in sound, dry wood many feet away from their origin, as long as they have a tube or some enclosed passageway to enable them to reach distant areas. Should the tube or passageway be removed, the isolated termites in the dry areas soon perish. Those below may, however, establish a new tube and continue to migrate at will. Termites have only a limited ability to exist in dry areas. Apparently they are able to condition their bodies while in their normal moist environment so that they may forage away from this environment for varying periods of time. But at all times it becomes necessary for them to return to their point of origin, or a similar environment, to enable their bodies to become rehabilitated for further work afield. As a result of this characteristic it was felt that the required optimum condition, so necessary to maintain life, be studied and duplicated in order that subterranean termites may be kept alive under captive conditions.

DEVELOPMENT OF TECHNIQUE.—A simple technique was developed by designing a moisture gradient tube. A casting plaster core was placed in the center of a glass tube 48 inches in length and 2 inches in diameter. The core was made by pouring casting plaster or cement into a rolled sheet of clear plaster to serve as a 1-inch diameter mold. As soon as the plaster was hardened it was peeled away and the core further dried under room conditions. The completed core, some 40 inches in length, was inserted into the glass tube and held in the center by means of a couple of wooden collars. The finished assembly was then placed on end in a petri dish where a ¼-inch water level was maintained by an inverted, edge-slotted, glass jar of water, fashioned after a "chicken fountain." The capillary rise of water up the plaster core soon created a moisture gradient. The bottom of the core was saturated, the upper portions becoming increasingly drier up to the point at which the plaster core extended beyond the capillary rise of moisture. Subterranean termites were then poured into the tube onto the plaster core and left to seek their optimum moisture requirement. It was soon learned that the termites congregated in great numbers at a point roughly 8 to 10 inches above the water line. While no food was provided the tube did serve to establish the point evidently best suited for the termites. The use of a soil moisture ohm meter unit, and small electrical soil moisture tensiometers, placed within the area of termite concentration, gave readings of the exact moisture requirement for subterranean termites. This reading indicated the termites prefer an atmospheric environment of 97.5% relative humidity.

Relative humidity readings of stations established at 6-inch intervals from the top of the capillary core to the bottom indicated percentages as follows:

Station 1	86
2	96
3	98
4	98½
5½	97½
6	97
7	97

It is interesting to note that the termites would invariably select the position established as station 5½ to concentrate their populations. This station, with a reading of 97½%, was selected in preference to any other, although the termites were given free range to select any other atmospheric condition afforded up and down the tube.

By duplicating the moisture gradient tube it became a simple matter to place portions of the wood on which the termites originally fed in the same area of termite concentration and thus provide them with the food required to sustain life. To date subterranean termites have been maintained in the laboratory under these conditions for more than 13 months and give every indication of continuing to thrive in a normal manner for an indefinite period.

Employing the method of the moisture gradient tube, a new and standardized method of studying subterranean termites in smaller populations was made possible. With this new approach one is able to study closely the feeding habits and general behavior and there is the added advantage of being able to quickly arrive at mortality determinations when treated wood is to be tested. It was discovered that the entire assembly could be prefabricated and placed in small standard 25×200 mm. test tubes. The value of employing small tubes lies in the convenience of storage and the ability to quickly select a stocked tube for study under the microscope. The mold for the core assembly of the small tube is made in this manner: A ¾-inch I.D. plastic tube 6½ inches long is cut down the middle so as to form two halves (see fig. 1); a small patch of Scotch tape is used to seal the ends and the trough-like mold is ready for pouring. Enough water is added to a small amount of casting plaster or cement to make it fluid. Before pouring, a thin coating of vaseline is rubbed on the inside of the mold to prevent sticking. The core is then poured until the mold is filled. A slight jarring of the

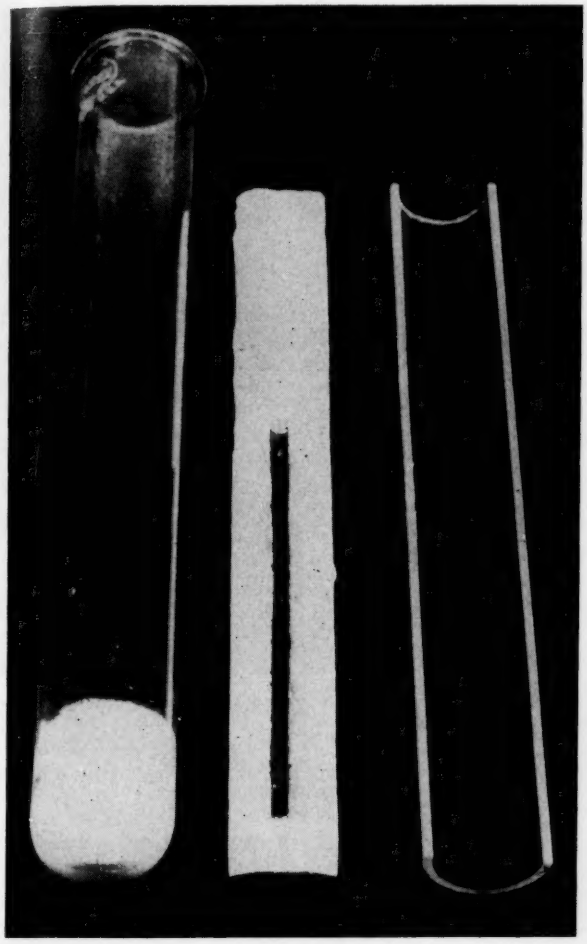

FIG. 1.—End-slotted test tube with cotton and plaster base, core assembly with imbedded black-dyed wood strip, and plaster mold used to cast core, make up the entire equipment for the prolonged maintenance of subterranean termites in the laboratory.

tube will settle the plaster or cement. The wood strips to be tested are then imbedded into the still-wet plaster to a depth of about $\frac{1}{8}$ inch, so that the greater portion remains above the surface. In this manner the plaster and wood are cast together. Wood strips should be made from clean, sound lumber and cut to lengths of $3\frac{1}{2}$ inches. They may be roughly $\frac{3}{8}$ inch high and $\frac{1}{8}$ inch thick.

The next step is to prepare the test tubes. These are first slotted in the ends by placing them against a narrow glass-cutting wheel, or the small holes may be made by heating and blowing a hole through the bottom of the tube. A small wad of absorbent cotton is then packed firmly in the tube end covering the hole or slot. A $\frac{1}{4}$-inch layer of casting plaster is then laid over the cotton and wetted to form a solid foot or floor with which the core end may make contact. The half-rounded core with impregnated wood strip is then slid into the tube and the completed assembly is now ready for operation. The tubes are placed on end in a beaker on the bottom of which is spread a thick layer of saturated cotton. The moisture is quickly absorbed through the slotted ends of the tubes and moves up into the cores by capillarity. The tubes are now ready to receive the termites. Once the termites are added, they will quickly seek the level best suited for their existence, and the tubes are then ready for observation (fig. 2).

STUDY OF FEEDING HABITS.—In keeping with this new technique it was felt that an opportunity was here presented to study microscopically the feeding habits of subterranean termites, particularly as there has been a question in the minds of some people as to whether termites are capable of ingesting sound wood that has not been "fermented." While it has long been common knowledge that termites do feed on sound wood, the feeding can now be studied in detail. To accomplish this objective the above-mentioned technique was employed with some modification. First, sound number one grade white pine lumber was selected. This was cut into strips as described above and put to soak in a 50% solution of Clorox to destroy any incipient organisms that might be present. Later the wood was boiled in distilled water to remove all traces of Clorox and ovendried overnight to remove all moisture. Next the wood was pressure-treated in pure India ink so that deep impregnation of the black ink was accomplished, then again oven-dried overnight to remove

FIG. 2.—Battery jars with saturated cotton at base which serves as reservoir for water supply to provide moisture which is taken up through the end-slotted test tubes. Termites may be seen on black-dyed and natural wood strips imbedded in plaster cores.

moisture. The plaster core was then molded and the sterile strip of black-dyed wood imbedded.

The termites selected for test were collected from sound white-wooded Douglas fir. Care was taken to use only the white-gutted forms from the sound parts of the wood. These were poured into the prepared tube and left to seek their own level. In 1½ hours the first of the termites started feeding on the black wood and traces of black wood could be clearly seen within the digestive tract when observed under the microscope. In 10 hours most of the termites were completely filled with black wood and the blackened abdomens could be clearly seen in sharp contrast against the white plaster of the core. Photographs were taken of the black-gutted termites along with some of the white-gutted forms in order to illustrate the comparison (fig. 3). In 12 hours all but a few of the termites were black-gutted, indicating the food was acceptable and consumed to a point where the original white food in the intestines was completely replaced. Later, when observed under the microscope, it was interesting to note the manner in which the termites fed upon the wood. The head would be slightly curved inward so that the mandibles could better grasp the tough fibers. With a gnawing motion of the mandibles and a twist of the head from side to side, firm fragments of wood fiber would be worried from the wood and quickly ingested. The black food could be easily traced as it entered the intestinal tract. Black fecal matter soon could be seen as it was deposited against the white core. The experiment clearly illustrates the time required to completely re-fill the stomach content with a new food.

DETERMINATION OF MORTALITY AND OTHER BIOLOGICAL FACTORS.—It became apparent that this technique was ideally suited for accurate mortality determination. When all test strips of wood are black-dyed and then treated with the various insecticides to be tested, a periodic examination of the termites definitely indicates whether the insecticide was ingested, whether it was effective merely as a contact poison, or whether the termites refused to feed and subsequently died of starvation. This presents a new opportunity to better understand the effects of insecticides on termites—whether they have a rapid lethal effect through contact only or are ingested to effect mortality at a later period. With this discovery we may now standardize by black-dying all wood prior to test so that we may know by actual observation the type of toxic action the various insecticides have on the termites.

Another observation resulting from the new technique of maintaining termites via the moisture gradient tube method is the interesting effect black and white wood has on the termites. Subterranean termites are considered to be cryptobiotic, that is, dark-seeking. They are also considered to be blind. However, while there is no evidence that they are capable of image perception it would seem that they are sensitive to the difference between black and white. We note they are quick to accept black-dyed wood in natural light when their climatic requirements are satisfied through the moisture gradient tube. The same wood, undyed and in its natural white state, is largely ignored. Yet when we place these white-wooded tubes in darkness, the termites commence feeding and continue feeding for at least 2 months, or until the food supply is exhausted. The storage of termites in darkened areas is recommended due to the fact that it provides the most favorable environment for prolonged maintenance. Further experimenting with dyed and undyed wood exposed to continuous artificial light indicates a definite preference for the black-dyed wood. Yet, as soon as the termites penetrate the surface dye, they continue to feed on the white wood beneath. The black-dyed wood appears to stimulate the immediate feeding, which continues after the black layer is consumed. Undyed wood, cut from the same source, is at first almost completely ignored until starvation forces a light feeding, which when once started continues in more normal manner.

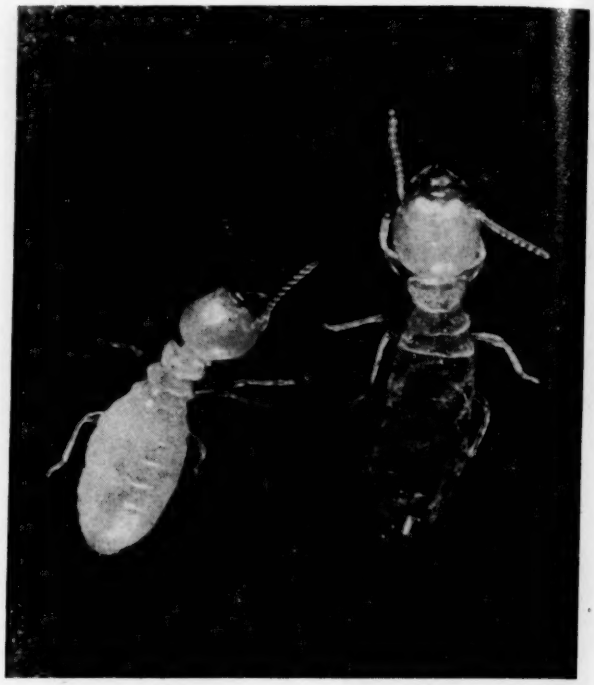

FIG. 3.—The western subterranean termite, *Reticulitermes hesperus*. Termite on left is white-gutted as a result of feeding on natural white wood. Termite on right clearly shows the black-stained gut after 10 hours of feeding on sound, sterilized black-dyed wood.

To further test this seeming preference of the termites for black-colored food, tightly rolled black and white photographic paper was used in place of wood. Again the feeding was more apparent on the black. Reversing the procedure, black-gutted termites were placed on pure white rolled photographic paper and white-gutted forms on black paper. The following morning nearly all termites placed on the black paper were gut-stained while those placed on the white were still black-gutted. An examination of both foods indicated feeding scars, but more were evident on the black than on the white. These experiments were conducted under constant artificial light.

Perhaps there are perceptive senses present that enable the western subterranean termite to differentiate between black and white. Continued study will have to be made to fully understand these processes. Meanwhile, the moisture gradient tube continues to serve as a means of further study of an insect about which much remains to be learned.

Habits of Attack by the Black Turpentine Beetle on Slash and Longleaf Pine in North Florida

RICHARD H. SMITH, *Entomologist, Forest Service, U.S.D.A.*

The black turpentine beetle, *Dendroctonus terebrans* (Oliv.), has in the past 6 years killed a large volume of pine timber in the south, from Texas eastward to Virginia and southward through central Florida. Because the insect rarely kills a large percentage of the stand at any one time, and because, to the untrained eye, it works rather inconspicuously, there is a tendency to minimize the extensiveness of its damage; but, by working consistently for the greater part of the year, it can attack as much as 20% of a stand in a single season, although 10% is much more common. The beetle is usually most active in disturbed stands such as logging areas, burned tracts, or naval stores operations. However, it can sometimes be found in undisturbed stands.

LIFE HISTORY.—The life history of the beetle is much like that of other bark beetles. The adult flies to a tree and bores through the outer corky bark and the inner spongy phloem. When it reaches the cambial layer on the face of the sapwood, the beetle bores downward instead of inward. Pitch mixed with frass and bark borings flows or is pushed out to the bark surface, where the characteristic pitch tube (fig. 1) is formed. An egg gallery $\frac{1}{4}$ to $\frac{3}{4}$ inch wide is formed as a result of the downward boring. Eggs are laid in a large linear group along one side of the gallery. Larvae hatch and bore away from the egg gallery, always staying in the phloem on the face of the sapwood. Pupation takes place in the same zone or a short distance outward in the corky bark. When maturation is complete, the adults bore out through the bark to complete the cycle.

Although its life history is in general similar to that of typical bark beetles, some of its habits are quite different from most of them. These habits, which are considered its vulnerable points, were determined from a series of ecological plots established and maintained in north Florida from 1952 to 1955. Although most of the data were collected from slash pine (*Pinus elliottii* Englm.) being worked for naval stores, adequate observations were made of slash pine without naval stores faces and of longleaf pine (*P. palustris* Mill.) with and without naval stores. The habits of the beetle were uniform on both the species and the conditions of the trees.

Differing from most bark beetles, the first attacks by the black turpentine beetle are few in number and low on the trunk portion of the tree (fig. 1, A). Attacks made during the first month are almost always confined to the basal 18 inches of the tree. These attacks, representing less than 20% of those which ultimately will be made on the tree, are in themselves seldom fatal. Over a period of 5 to 7 months the attacks slowly increase in number and height on the tree (fig. 1, B, C, and D). Although the beetle is active all year in north Florida and south Georgia, 90% of its attacks are made from April to October. Diameter has a positive correlation with the number of attacks, but has little effect on the height of them.

INITIAL ATTACKS VERSUS TOTAL ATTACKS.—Nine hundred and fifty trees were observed as they became attacked. Counts were made of the number and location of these attacks during the first month. These data show an average of 4.7 attacks per tree during the first month; figure 1,A illustrates the usual situation at the end of the first month. For location references, the tree trunk was divided into four height sections. Starting from the ground line, these were: 0″ to 18″, 18″ to 36″, 36″ to 72″, and above 72″. The location-distribution of these first months' attacks was 4.4—0.3—0—0; or, by percentage, 94—6—0—0, respectively. The few attacks made above 18 inches during the initial month can be assigned to the latter part of the period. This was determined by a smaller sampling of trees at biweekly intervals and by the age-color changes of the pitch tubes. For the first 2 to 4 weeks the pitch tubes have a fresh, clear, whitish-red color. With age, they turn to dull grayish-red. Also, when trees with less than four attacks were observed these attacks were always below 18 inches and were quite often within 6 inches of the ground. Thus the evidence is fairly conclusive that attacks are not made above 18 inches until attacks are made below this height.

Of the original 950 trees, 227 were allowed to continue for the duration of the attacks, which resulted in the death of the tree. The final average number of attacks for these trees was 28.6, with a distribution of 18.4—6.1—3.4—0.7, respectively, in the four designated height zones by percentage, this distribution was 64—21—12—3. Figures 1, C and D illustrate the appearance of the tree at the conclusion of the attacks. Table 1 gives the data for the 227 trees.

A larger sample of 496 trees, of which the 227 were a part, was also recorded for the number and location of the total attacks on the tree. The data show a percentage distribution of 59—25—14—2.

It is noteworthy that in the case of both the initial and total attacks the smaller sample of 227 trees was almost identical with the larger sample for both the number and distribution. Another observation was that there was little variation between the 9 plots and 3 years in which the data were collected.

As a matter of speculation, it is thought that attacks above 18 inches are made by beetles which first reach the tree below the 18-inch height. Complete preventive protection was secured by spraying only the basal 18 inches of trees. Where trees were already attacked, a spray applied to the height of the highest attack prevented subsequent attacks in and above the sprayed zone. Thus there are strong indications that attacks above 18 inches are dependent on attacks below this height, or are made by beetles which first reach the tree below 18 inches.

MONTHLY HEIGHT DISTRIBUTION OF ATTACKS.—Two hundred fifty-six trees were observed monthly for the duration of the attack. Data obtained from the study are shown graphically in figure 2, in which the cumulative

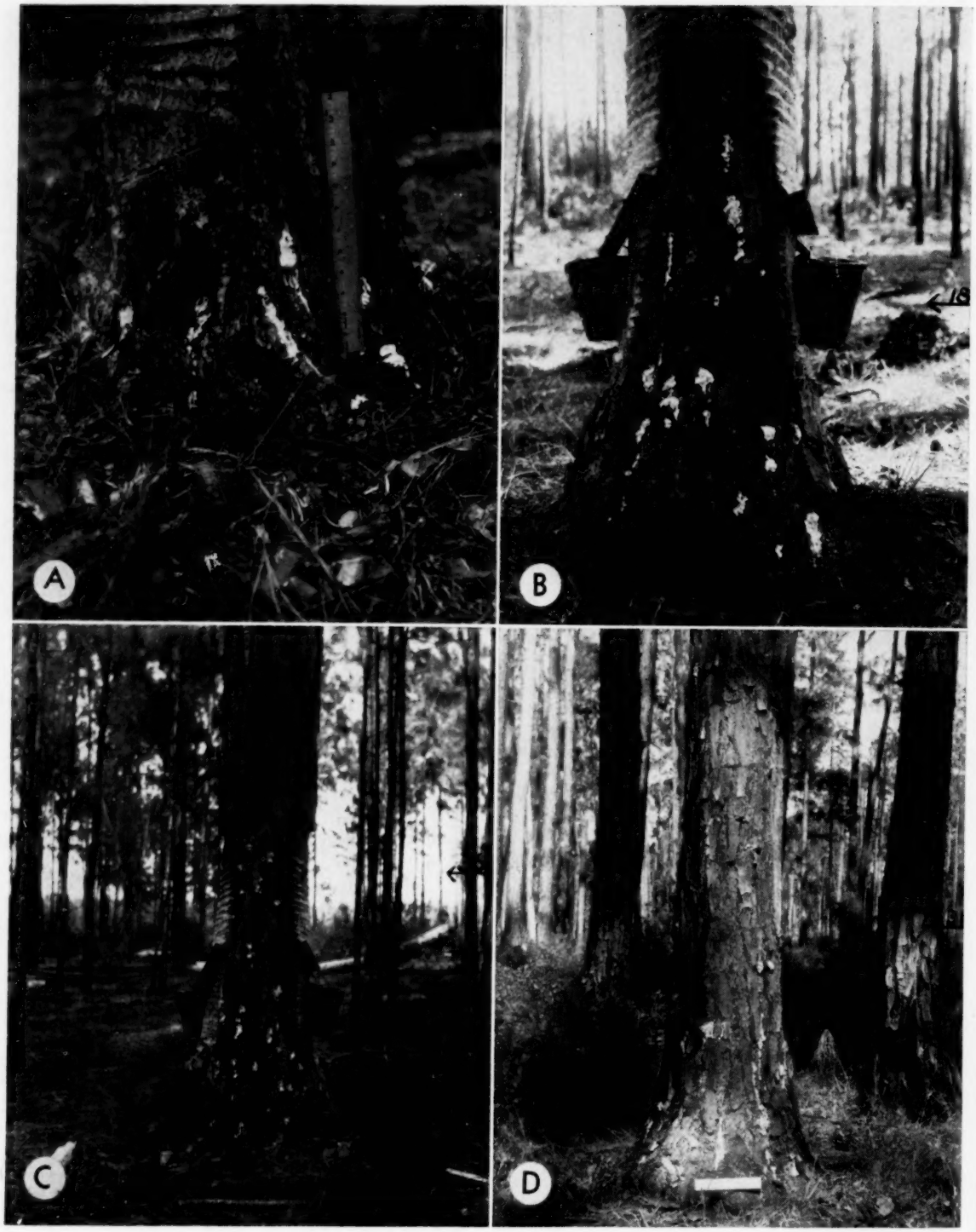

FIG. 1.—Progress of black turpentine beetle attacks on slash pine. *A*, First attacks are made below an 18-inch height. *B*, After these first ones, attacks are made above an 18-inch height. *C*, In time some attacks are made above 36 inches but they are still concentrated below this height. *D*, Eventually a few attacks may be made above a 72-inch height.

number of attacks is plotted over time for each of the established four basal zones of the tree. The time ordinate is the months in which fresh attacks were made following the initial attacks. There was a high degree of consistency between the 3 years and the 11 plots.

It is apparent from the graph that the attack reaches its peak during the second and third months, with a gradual tapering off until the seventh month. A few trees received attacks over a period of more than 7 months, but these were too few to be considered; likewise, some

Table 1.—Black turpentine beetle: Number of attacks made during the first month versus the final number of attacks.

Plot No.	Number of Trees	First Month's Attacks					Total Attacks				
		Total No.	Below 18″	18″–36″	36″–72″	Above 72″	Total No.	Below 18″	18″–36″	36″–72″	Above 72″
			Per Cent					Per Cent			
1	7	34	94	6	0	0	207	62	23	14	1
2	20	112	94	6	0	0	579	69	18	11	2
3	16	97	87	12	1	0	481	57	23	15	5
4	15	46	89	11	0	0	548	55	25	15	5
5	16	61	89	11	0	0	422	63	24	12	2
6	13	56	91	9	0	0	319	66	17	15	2
7	20	68	97	3	0	0	580	61	23	15	1
8	36	206	97	3	0	0	981	63	22	13	2
9	84	489	96	4	0	0	2386	69	20	9	2
Total or per cent	227	1169	94	6	0	0	6503	64	21	12	3
Average No. of Attacks per Tree		5.2	4.9	0.3	0	0	28.6	18.4	6.1	3.4	0.7

trees did not receive fresh attacks in all 7 months. It should be mentioned that the months were those in which fresh attacks were registered on the tree after the initial attacks had been made. They are not chronological months, since it is not unusual to have a month pass by without fresh attacks.

It is clearly shown that the attacks are scattered over a 5- to 7-month period; that the total attacks are concentrated in the basal 36 inches of the tree; and that the greatest number of attacks are made during the second and third months. The per cent distribution of the attacks for the first to the seventh month, respectively, was: 19, 29, 27, 14, 6, 3, and 2.

PERIOD OF YEAR IN RELATION TO THE INCIDENCE OF ATTACK.—An important characteristic of the beetle with respect to its control is its year-round activity in the naval stores belt of Florida and Georgia. The timberland owner's primary defense is persistent surveillance and action.

For 3 years, 1953–55, data were taken on the relative activity of the beetle. All years showed the general pattern illustrated in figure 3. It is apparent that the beetle is active to some degree all year long, with 90% of its action occurring from April to October. This graph also illustrates a common habit all 3 years: the surge of activity in March and April. Thus these months should be watched as indicators for the season's activity.

The graph also illustrates that both the number of attacks and the incidence of initially attacked trees are closely parallel.

TREE DIAMETER IN RELATION TO ATTACK.—Diameter might influence the number and location of attacks. From measurements in table 2, it is apparent that there is a positive correlation between the number of attacks

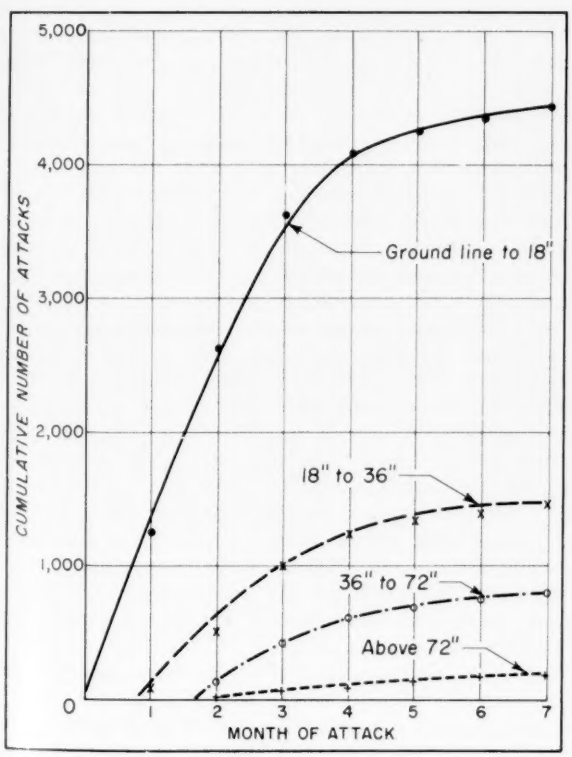

FIG. 2.—Time-height distribution of attacks on 256 slash pines in north Florida.

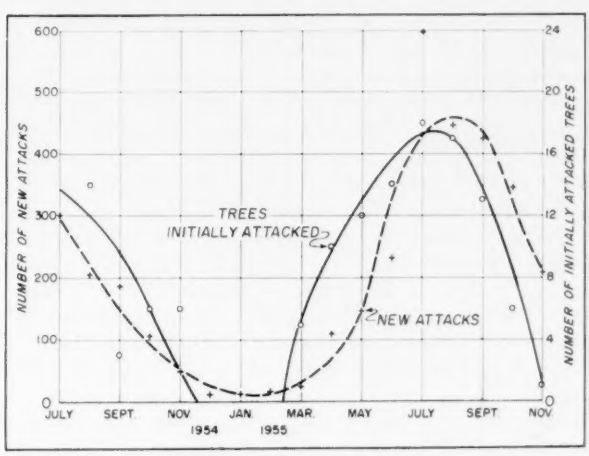

FIG. 3.—Monthly incidence of attacks and of initially attacked trees, Putnam County, Florida, experimental area.

Table 2.—Black turpentine beetle: the effect of diameter on the number and location of attacks.

Diameter Class (Inches)	No. of Trees	Per Cent				Average Attacks per Tree
		Below 18″	18″–36″	36″–72″	Above 72″	
5	6	98	2	0	0	6
7	35	73	21	6	0	10
9	32	65	24	10	1	13
11	48	65	24	10	1	18
13	48	63	25	11	1	19
15	43	63	24	12	1	20
17	22	54	29	14	3	27
19	10	47	25	20	8	35
21	3	42	26	20	12	41

and diameter. But, with the exception of the extremes in diameter (i.e., below 9 inches d.b.h. and above 17 inches d.b.h.), there is little correlation between the location of the attacks and diameter.

SUMMARY.—The black turpentine beetle, *Dendroctonus terebrans* (Oliv.), has caused extensive losses in pine timber in the southern states. In many respects it is a typical bark beetle; however, some of its habits are quite different. These habits must be considered in developing control recommendations.

An average of five attacks were made on a tree during the first month. These attacks were confined to the basal 18 inches and comprised less than 20% of the total number made on a tree.

In the months subsequent to the initial one, the attacks increased in number and in height on the trunk of the tree. An average of 28 attacks were eventually made on the tree; 85% of these were in the basal 36 inches of the trunk, while only 3% were above a height of 72 inches.

It is speculated that attacks above 18 inches, which were made only after there were attacks below this height were either dependent on the lower attacks or made by beetles which first contacted the tree in the basal 18 inches.

The attacks were spread out over a period of 5 to 7 months, with a peak being reached during the second and third months. Although the beetle was active all year long in the naval stores belt of Georgia and Florida, 90% of its attacks were made between April and October.

There was a positive correlation between tree diameter and the number of attacks, whereas the height distribution of the attack was unaffected by diameter on trees between 9 and 17 inches d.b.h.

Tests with Dow ET-57 against Cattle Grubs in Oregon[1]

A. R. ROTH and GAINES W. EDDY,[2] *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

The use of chemicals as systemics for the control of insects attacking both plants and animals is gaining considerable attention. The kill of insects by internal administration of insecticides to animals has been accomplished by several workers. Knipling (1938) showed that the manure of cattle fed phenothiazine was toxic to horn fly larvae. Bruce (1939) obtained similar results with zinc oxide and certain other chemicals. Lindquist *et al.* (1944) and Knipling *et al.* (1948) showed several insecticides to be active as chemotherapeutic agents against certain bloodsucking parasites. deToledo & Saur (1950) were perhaps the first to demonstrate the systemic effect of an insecticide against the warble fly,[3] *Dermatobia hominis* (L., Jr.). McGregor *et al.* (1954) showed that some of the organic phosphorus compounds were very active as systemics against the common cattle grub (*Hypoderma lineatum* (De Vill.)). The same authors (1955) demonstrated dieldrin, aldrin, and lindane acted as systemics against cattle grubs (*lineatum* and *bovis* (L.)) and certain other livestock pests, and Roth (1955) also showed dieldrin to be systemically active against cattle grubs.

In tests conducted during March 1955 the authors found that Dow ET-57 caused good kill of cattle grubs (*bovis*) present in the backs of animals when administered orally at 50 and 100 mg/kg. Research during the summer of 1955 by workers at the Kerrville, Tex., laboratory of the Entomology Research Branch indicated the material to be effective in preventing grubs (*lineatum*) from reaching the backs of animals. A preliminary report on the effectiveness of the material as a systemic against cattle grubs was presented by Lindquist (1956). Additional experiments with this material as a systemic and as a spray against cattle grubs are reported herein.

SYSTEMIC TESTS.—The experimental cattle were of several breeds, and most of them were yearlings. They were weighed before treatment either on a portable animal scale or on the scales used by the local livestock auction. The animals were treated with ET-57 orally or subcutaneously at doses ranging from 10 to 200 mg./kg. In the oral treatments the technical material was administered in a capsule with a balling gun. Subcutaneous injections were made with a 10% solution of ET-57 in polyethylene glycol (Carbowax 400). Only freshly prepared solutions were used.

Observation were made one to three times weekly over a period of 12 weeks on the kill of grubs present in the backs of the cattle at the time of treatment as well as on the encystment of new ones. Those attaining maturity were captured by gluing small perforated plastic containers over the cysts. The captured grubs were held at room temperature in individual glass vials containing moistened sand for pupation and emergence as adults.

Effect of encysted grubs.—The results of the tests against second- and third-instar grubs encysted in the backs of cattle are presented in table 1. The table includes only observations made during the first 4 weeks and every 2 weeks thereafter.

Fair but incompete kill of grubs was caused in the

[1] Accepted for publication October 11, 1956.

[2] R. A. Hoffman assisted in some of these experiments.

[3] Human bot fly is the common name adopted by the Entomological Society of America.

Table 1.—Effectiveness of Dow ET-57 as a systemic treatment against cattle grubs.

Dose (Mg./Kg.)	Species	Number and Instar of Grubs		Per Cent Mortality (Cumulative) at Indicated Weeks After Treatment[a]								Number of Pupae	
		Second	Third	1	2	3	4	6	8	10	12	Collected	Emerging as Flies
10	*lineatum*	7	3	0[b]	0	0	0[b]	0	60	80	80	1	0
	bovis[c]	5	49	39	58	65	66	66	66	—	—	2	1
	bovis[c]	0	8	0	0	0	38	38	38	—	—	—	—
25	*lineatum*	0	7	0	0	0	0	14	14	14	14	1	1
	lineatum	10	0	0	30	100	—	—	—[b]	—	—[b]	—	—
	bovis	0	5	60	60	60	60	60	60	—	—	1	1
	lineatum[c]	3	23	0	0	69	88	92	92	92[b]	92	1	1
	bovis[c]	0	11	0	55	55	55	73	73	73	—	1	0
	bovis[c]	0	25	28	36	48	48	48	—	—	—	7	1
50	*bovis*	0	8	0	0	0	0	13	13	—	—	1	0
	bovis	9	13	0	9	50	95	—	—	—	—	—	—
75	*lineatum*	7	3	50	70	90	100	—	—	—	—	—	—
	lineatum	2	0	100	—	—	—	—	—	—	—	—	—
100	*lineatum*	19	11	63	80	90	100[b]	—	—	—	—	—	—
	lineatum	8	6	28	35	71	71	86	86	86	—	2	0
	bovis	0	24	25	25	29	38	38	—	—	—	12	2
	bovis	1	6	42	85	85	85	—	—	—	—	—	—
200	*lineatum*	19	0	100	—	—	—	—	—	—	—	—	—
	lineatum	0	51	0	35	88	94	94	94	94	94	3	2

[a] Grubs not accounted for emerged from host.
[b] New grub encysted, species not determined.
[c] Treatment administered subcutaneously; other treatments given orally.

three animals treated at 10 mg./kg. One of six treatments at 25 mg./kg. caused 100% kill, whereas in another animal the kill was only 48% over a period of 6 weeks. On the basis of these limited data it appears that ET-57 acts systemically on grubs when injected subcutaneously. Two animals treated at 50 mg./kg. showed incomplete kill, but 100% mortality was caused in the two animals treated at 75 mg./kg. However, complete kill was caused in only one of the four animals treated at 100 mg./kg. and in one of the two treated at 200 mg./kg.

Although the variation in effectiveness may have been due to a number of factors, it was believed to be attributable largely to differences in the number and/or age of the grubs present at the time of treatment. In most tests the best results were obtained in animals infested chiefly with second-instar grubs and the poorest kills in those having more third-instar grubs.

Although these data indicate that the material is more efficient in killing young grubs, some of the incomplete kills were no doubt due to the fact that many of the grubs were too near the point of emergence from the host when the treatments were administered. The various observations indicate that the material cannot be expected to cause complete kill of grubs nearing maturity. For example, three pupae were collected from one of the animals 2 to 3 weeks after it had been treated with 200 mg./kg., and normal flies emerged from two of them.

Effect on grubs prior to encystment.—Three herds of cattle were treated in December 1955 prior to the appearance of grubs in the backs. The animals were practically all yearlings and were weighed just before treatment. No grubs were removed from them during the tests except for identification purposes. Herd No. 1 consisted of 14 Herefords and 6 Anguses, divided into three groups composed of about the same number of each breed. The animals were infested principally with *lineatum*. Herd No. 2 consisted of Herefords only and appeared to be about equally infested with *lineatum* and *bovis*, and herd No. 3 was made up of Holsteins, infested only with *bovis*. Grub counts were made once or twice each month, and the averages are presented in table 2.

In herd No. 1 grubs appeared in 7 of the 8 animals treated at 50 mg./kg., in 5 of the 7 treated at 100 mg./kg., and in all 5 of the control animals. The largest number of grubs encysting in the back of any animal was 21 for the group receiving the 50-mg., 7 for those receiving the 100-mg. dose, and 38 for the control group. Although some grubs did appear in the animals treated at 100 mg./kg., the average number per animal was less than in those treated at 50 mg./kg. and far less than in the controls. The highest counts were made in February. The number of grubs present during January indicated that the larvae had left or were about ready to leave the gullet area when the treatment was administered. This may have influenced the results.

Although herds Nos. 2 and 3 were not heavily infested, no grubs encysted in the backs of the animals treated at 100 mg./kg., whereas a fair number appeared in the

Table 2.—Effect of oral administration of Dow ET-57 on the encystment of cattle grubs.[a]

Dose (Mg./Kg.)	Number of Animals	Average Number of Grubs per Animal				
		January	February	March	April	May
		Herd No. 1				
100	7	1.0	1.1	1.0	0.4	0
50	8	3.2	8.7	6.4	1.5	0
Controls	5	6.8	13.3	13.2	4.2	0.4
		Herd No. 2				
100	7	0	0	0	0	0
25	8	0	1.0	2.2	2.4	1.5
Controls	6	0.5	0.5	1.6	3.5	3.3
		Herd No. 3				
100	5	0	0	0	0	0
50	5	0	0.2	0.2	0.2	0.2
Controls	5	0	1.8	7.6	6.6	5.5

[a] Treatments given in December prior to appearance of grubs.

control animals as well as in those receiving the smaller doses.

These data indicate that ET-57 is effective in preventing grubs from reaching the backs of cattle. However, further research is necessary to determine whether the material may be even more efficient if administered earlier than was done in these experiments.

SPRAY TESTS.—In tests to determine the effectiveness of ET-57 when applied as spray on the backs of cattle, three animals infested with *bovis* were treated. A 3-gallon compressed-air sprayer was used to apply 2 quarts per animal. A laboratory-prepared concentrate containing 50% of ET-57 in xylene and Triton X-100 was used. One of the animals was sprayed with a 1% and two with a 2.5% concentration. The animal sprayed with 1% had 8 grubs and of the other two, one had 17 and the other 6 grubs. No grubs were killed by the 1% spray over a period of 14 days. On the animals sprayed at 2.5%, the kill ranged from 50 to 65% after 7 days and was 100% after 14 days. It is possible that the insecticide was absorbed by the animals and destroyed young grubs in the body, but no data to confirm this conclusion were obtained.

In another experiment 15 yearling Herefords infested with an average of 6 grubs (*bovis*) each were treated with a 1% spray prepared from a 25% wettable powder. The spray was applied at approximately 250 pounds' pressure at the rate of about 3 quarts per animal. About half the grubs were taken out 6 days and the remaining grubs 13 days after treatment. Only 25 and 53% mortality were indicated at the respective examinations.

SUMMARY.—During 1955–56 Dow ET-57 was tested as a systemic against cattle grubs (*Hypoderma lineatum* (De Vill.) and *bovis* (L.)) at doses of 10 to 200 mg./kg. This material was found to be effective against grubs present in the backs of animals as well as against the encystment of new ones. Second-instar grubs were killed more rapidly and efficiently than third-instar grubs. The material failed to kill many of the grubs that were within 2 weeks of emergence.

In limited spray tests the material caused 100% kill of grubs at 2.5 but only about 50% or less at 1% concentration.

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Tests with Dow ET-57 against Two Species of Cattle Grubs[1]

W. S. MCGREGOR[2] and R. C. BUSHLAND,[3] *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

A number of compounds have been administered to cattle and found effective as systemic insecticides against larvae of *Hypoderma lineatum* (De Vill.) and *bovis* (L.) after the grubs have appeared in the back. Tests with some phosphorus insecticides were reported by McGregor *et al.* (1954) and with some chlorinated hydrocarbon insecticides by McGregor *et al.* (1955). Those results were discouraging because none of the treatments seemed to have any effect on first-instar larvae. New grubs continued to encyst in the back and, as the grub season lasts several months, none of the chemicals showed promise as a one-treatment control. Recently a new phosphorus insecticide, Dow ET-57, has shown promise in killing cattle grubs in all three instars. Tests at Kerrville, Tex., with this new chemical are reported herein.

PRELIMINARY TESTS.—Early in the spring of 1955 Dow ET-15 and ET-57 were submitted to the Kerrville, Tex., and Corvallis, Ore., laboratories for testing. Both compounds were screened at Kerrville by the routine procedure (McGregor & Bushland 1956), dosing infested guinea pigs at the rate of 25 mg./kg. ET-57 had no effect on the screw-worms (*Callitroga hominivorax* (Cqrl.)), nor did it kill stable flies (*Stomoxys calcitrans* (L.)) or lone star ticks (*Amblyomma americanum* (L)) which engorged on the treated animals. ET-15 was lethal to all three pests, both subcutaneously and orally, and seemed a more promising material.

In simultaneous studies at Corvallis, Roth & Eddy

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[2] Resigned July 13, 1956.

[3] Wilford Paynter, Hyde County agricultural agent, Highmore, S. Dak., selected the grub-infested South Dakota cattle. W. R. Pringle, superintendent of the Highmore substation of the South Dakota State College Experiment Station, provided facilities for weighing and treating the test animals. J. M. Dreiss and M. O. Pickens, biological aids of the Entomology Research Branch, assisted in the experiments. The Texas Agricultural Experiment Station provided for the slaughter and examination of two cattle in the Animal Husbandry Department of Texas A. & M. College.

(1956) found that ET-57 showed systemic effects against second- and third-instar larvae of *H. bovis*, but ET-15 did not. On the strength of the Corvallis data we included ET-57, along with ET-15, in a series of chemicals being given to groups of five Hereford calves in June 1955. The treatments were administered by cooperating veterinarians of the Animal Disease and Parasite Research Branch, U. S. Department of Agriculture, and their clinical observations are reported separately (Radeleff & Woodard 1957).

The 10 calves treated with ET-57 and ET-15 were from the herd of the U. S. Dept. of Agriculture at Camp Stanley, Tex., and had been similarly exposed to natural infestation by *lineatum*. In August grubs began to appear in the five calves treated with ET-15 and continued to encyst through September and October. At the final examination in November they were infested with a total of 98 grubs, the individual counts being 11, 16, 17, 19, and 35. Of the five calves treated with ET-57, four had one grub each appear in the back and one animal remained grub-free. All these larvae died in the second instar, whereas most of the 98 in the ET-15-treated calves completed their growth.

Table 1.—Grub infestation in cattle drenched with 100 mg./kg. of Dow ET-57 on February 10, 1956, and in untreated cattle.

Animal No.	Number of Grubs		New Grubs Feb. 17 to June 10	Total Number of Grubs	
	Feb. 17	Feb. 24		Died	Survived
Emulsion-treated cattle					
1351	25	0	0	25	0
1352	16	6	0	14	2
1358	10	0	0	10	0
1359	30	2	0	28	2
1361	13	0	0	13	0
1363	2	2	0	1	1
1364	53	14	0	48	5
1369	19	2	0	17	2
1370	22	9	0	17	5
1371	19	3	0	17	2
1372	18	1	0	17	1
1375[a]	32	6	0	28	4
1376	19	1	0	18	1
1377	11	1	0	10	1
1380	8	0	0	8	0
1381	15	2	0	14	1
1387	19	5	0	17	2
1391	38	5	0	34	4
1392	6	2	0	4	2
1393	8	0	0	8	0
Average	19.2	2.8	0	17.4	1.8
Suspension-treated cattle					
1337	15	0	0	15	0
1338	13	1	0	12	1
1339	26	1	0	25	1
1340	5	0	0	5	0
1341	16	1	0	15	1
1342	9	0	0	9	0
1343	5	0	0	5	0
1353	25	0	0	25	0
1360	12	2	0	10	2
1366	21	2	0	21	0
1378	33	9	0	26	7
1383	23	9	0	15	8
1385	17	2	0	15	2
1389	8	0	0	8	0
1394	10	0	0	10	0
Average	15.9	1.8	0	14.4	1.5
Untreated controls					
1344	45	57	29	4	70
1345[a]	22	25	13	5	30
1346	33	44	19	7	45
1347	13	23	36	4	45
1348	58	81	33	7	84
1349	18	38	36	3	51
1350	22	36	18	7	33
1354	23	33	33	4	52
1355	1	10	50	0	51
1356	22	33	36	1	57
1357	19	21	7	4	22
1362	27	42	42	8	61
1365	39	44	20	6	53
1367	16	24	11	10	17
1368	43	53	28	2	69
1373	25	29	53	2	76
1374[b]	61	93	70	14	117
1379	41	64	40	6	74
1382[b]	65	104	114	29	150
1384	49	78	39	5	83
1386	33	62	45	3	75
1388	25	30	29	6	28
1390	18	43	31	7	42
1395	32	34	5	8	29
1396	30	32	13	9	33
Average	31.2	44.6	34.0	6.4	57.9

[a] Animal slaughtered.

[b] Figures after Feb. 17 are estimates; grubs were too numerous for accurate charting.

(*Continued in next column*)

Laboratory Tests With Guinea Pigs.—As the routine screening procedure had not detected a chemical which looked promising against cattle grubs, special tests were made with ET-57. The compound dissolved in Tween 20 (polyoxyethylene sorbitan monolaurate) was given both orally and subcutaneously to guinea pigs at doses greater than the 25 mg./kg. which had failed in the first trial.

Day-old screw-worm larvae in artificial wounds on the guinea pigs were killed within 3 hours of each treatment at 100 mg./kg. Stable flies fed on their shaved bellies 1 and 4 hours after treatment were killed. At 24 hours after treatment there was only partial mortality, and at 48 hours the guinea pigs seemed no longer toxic to stable flies.

When the chemical was administered at 50 mg./kg., results were variable. In one test both oral and subcutaneous doses caused death of the screw-worm larvae within 24 hours and stable flies fed on treated animals were also

killed. When the treatment was repeated on two more guinea pigs, the screw-worm larvae seemed affected but recovered. In a further test with a 25 mg./kg. dose against stable flies and screw-worms both oral and subcutaneous treatments were ineffective.

It seems that the routine screening procedure failed to detect ET-57 merely because an insufficient dose was given. Although many compounds have shown insecticidal effects at doses of 10 mg./kg. and less, the screening procedure for new compounds has been amended to include observations at 100 mg./kg.

TESTS WITH SOUTH DAKOTA CATTLE.—To avoid waiting until the summer of 1956 for further practical observations during the cattle grub season in Texas, a special experiment was made with cattle purchased in South Dakota. In February 1956 calves were purchased from an untreated herd near Highmore, which had been grossly infested with both *H. bovis* and *lineatum* the preceding year. The calves had been born in March or April 1955, and at the time of purchase weighed an average of 379 pounds. The experimental group consisted of 20 steers and 40 heifers.

The cattle were trucked from the ranch to the Highmore substation of the South Dakota Agricultural Experiment Station, where they were held for 2 days to become adjusted after the move. They were then weighed individually (Feb. 10) and given an appropriate amount of 5% ET-57 for a dose of 100 mg./kg. with a 4-ounce drenching gun. A suspension of 25% wettable powder was given to 5 steers and 10 heifers, and 5 steers and 15 heifers were drenched with an emulsion made from a concentrate containing 25% of ET-57, 65% of xylene, and 10% of Triton X-100. Both concentrates were diluted with 4 parts of warm tap water. Ten steers and 15 heifers were left untreated to serve as controls. The calves were held at the Experiment Station feed lot for 4 days, and then loaded on trucks and transported to Kerrville.

In South Dakota, where the cattle were treated, the weather was so cold that it was not practical to make a thorough examination of them. On their arrival in Kerrville on February 17, the heavy winter coat was clipped from the backs of the animals so that grub infestations could be more accurately determined. Beginning on this date they were examined at weekly intervals until June 10. A chart was made for each calf, and the position of each grub was noted as it was detected. The records are summarized in table 1.

At the first examination all the calves were infested. The 20 drenched with the emulsion had an average infestation of 19.2, the 15 drenched with the suspension 15.9, and the 25 untreated animals 31.2 grubs per animal. The insecticidal effects of the treatment reached their full expression by February 24. Most of the grubs that survived for 2 weeks were able to complete their development. There seemed to be no difference between steers and heifers.

Except for the effort to divide the steers and heifers into three groups, the South Dakota cattle were selected entirely at random, as the weather was too severe and the hair of the animals too long for selection on a basis of apparent infestation. The observation that the untreated cattle had almost twice as many grubs as the treated ones upon arrival in Kerrville is explained on the theory that they were probably equally infested at the time of drenching but effects were already shown after the first week.

The presence of a newly migrated larva in the back was noticeable only as an inconspicuous bump, and such bumps could be expected to disappear soon after the death of the larvae. The presence of larger larvae, either dead or alive, could be detected 1 week after treatment, but after 2 weeks only living larvae remained readily noticeable. Some of the dead larvae protruded through the hole in the hide; others could be detected only as the skin opening began to heal.

New grubs continued to appear in the control animals, and the infestation reached a peak of 61.5 per animal on March 30. No new grubs appeared after the end of April. The final examination was made on May 11. When considerable numbers of larvae had completed their growth, 16 of the most heavily infested control animals were confined in concrete-floored stalls, where the dropped insects could be collected. As usual, *lineatum* predominated among the early larvae, but those that dropped during the last month of observation were almost entirely *bovis*.

Two of the heifers were slaughtered on March 20. When the untreated control heifer (No. 1345) was skinned, 29 live grubs were found encysted. When examined on March 16, 27 live grubs had been recorded. Earlier one grub had migrated from the animal. The emulsion-treated heifer (No. 1375) had 32 grubs in her back on arrival in Kerrville on February 17. On February 24 there were six grubs; on March 2, five; on March 9, four; and by March 16 those four grubs had migrated and were counted as survivors. When this animal was skinned on March 20, 54 shriveled, dead grubs were found underneath the hide. Some of those grubs had apparently died before the first examination, as there was evidence of only 32 grubs at that time. At the time of slaughter no grubs could be found in the gullet of either animal.

The average number of grubs surviving in the 35 treated cattle was 1.6, there being little difference between treatments with emulsions and suspensions. Comparison of that figure with the average of 57.9 for the controls suggests that the grub mortality due to treatment averaged 97.2%. Although new grubs continued to appear in the control animals, so that during the observation period the average population increased from 31.2 to 57.9 per animal, not a single new grub, either *lineatum* or *bovis*, was found in any of the treated cattle.

Thus, in preventing hide and meat damage the treatment was 100% effective. Roth & Eddy (1956), using ET-57 from the same batch as that given the South Dakota cattle, also reported good results. Further work is required to establish a dose that will work with certainty to prevent grubs encysting in the backs of treated cattle.

From the results with the South Dakota cattle it seems that the drenching was effective against all grubs within 10 weeks of migrating to the back. Further work should be done to establish how early in the life cycle the treatment is effective.

SUMMARY.—Dow ET-57 shows promise as a systemic insecticide for livestock pests. In laboratory tests the compound was toxic to screw-worms (*Callitroga hominivorax* (Cqrl.)) and stable flies (*Stomoxys calcitrans* (L.))

when given orally or subcutaneously to guinea pigs at 100 mg./kg. It was less effective at 50 mg./kg. and ineffective at 25 mg./kg. When administered orally at 100 mg./kg. to five Hereford calves presumably infested with *Hypoderma lineatum* (De Vill.), it seemed effective, as only four larvae appeared and they died in the second instar. A comparable group of five calves treated with ET-15 had 98 larvae develop.

ET-57 administered orally at 100 mg./kg. to 35 South Dakota calves naturally infested with *H. bovis* (Deg.) and *lineatum* caused an estimated 97.2% control and completely prevented encystment of new larvae.

Before ET-57 can be considered for practical control further research is required.

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Toxicological Studies of Dow ET-57 in Cattle and Sheep[1]

R. D. Radeleff and George T. Woodard, *Animal Disease and Parasite Research Branch, Agr. Res. Serv., U.S.D.A.*

Dow ET-57 has been found by McGregor & Bushland (1957) and Roth & Eddy (1957) of the U. S. Department of Agriculture and Wade & Colby (1955) of the Dow Chemical Company to be systemically effective against the cattle grubs *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.) and against certain other parasites when administered orally. To determine the acute toxicological hazard involved in the use of this compound, the studies discussed in this paper were carried out.

Experimental Compound.—During the initial phases of the entomological studies, a technical *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate[2] was furnished. A second compound was later supplied as Dow ET-57, which was less toxic to cattle than the earlier material furnished. To avoid misunderstandings, which would inevitably result from reporting research with both materials, the data presented in this paper are only those developed with Dow ET-57.

Dow ET-57 was furnished by the Dow Chemical Company as a wettable powder 25% material of exceptionally good suspensibility. Suitable water suspensions of this wettable powder were made for administration to the animals.

Animals.—The cattle used in these studies represented a cross section of available cattle. Some were grade cattle purchased through public auction, some were from the Government herd, and some were high-grade or purebred cattle on cooperating ranches. All were in good health, although they ranged considerably in degree of fatness. The sheep were from our own Government flock and were in good health and condition. Cattle and sheep held at the laboratory were fed alfalfa hay. All others were maintained on the range.

Methods.—Dow ET-57, in all cases, was administered orally as a suspension, using dosing syringes. Each animal maintained at the laboratory was weighed before treatment to establish the proper dose. The weights of animals treated for cooperating ranchers were usually estimated. Observations were made daily, and, if indicated, at night, to establish the full chain of symptoms displayed.

Erythrocyte cholinesterase was determined by the electrometric method of Michel (1949) before treatment and at intervals thereafter.

Oscillographic studies were made of the heart action in one sheep, using a Grass preamplifier and a DuMont oscilloscope.

Results.—*Cattle.*—The results of treatments of cattle at the laboratory are summarized in table 1, which gives the gross results, and in table 2, which presents a summary of the cholinesterase determinations made. Dosages of 125 mg./kg. or higher were toxic to yearling and older cattle. No symptoms were noted in any of the age groups at 100 mg./kg. except that one calf 4 months of age showed a mild diarrhea.

In addition to the cattle treated at the laboratory, 208 cattle were treated for cooperating ranchers. This group included 25 calves 4 to 6 months of age, 23 calves 6 to 12 months of age, 25 yearling cattle, and 135 older cattle. In treating this large group, the weight of each animal was estimated by three men and the average of their estimates was used to calculate the dose to be administered. All were dosed at 110 mg./kg. based on this average of estimates. From preliminary studies we determined that such a procedure would yield weights varying $\pm 10\%$ from the true weight in extreme cases, with most of the averages being within $\pm 5\%$ of the true weight. In this manner the dosage given these cattle actually ranged from approximately 100 to 120 mg./kg.

Each rancher was apprised of the symptoms of poisoning and under the cooperative agreement was free to make claims for damage if he felt there was any. No claims were made, nor was any deviation from normalcy noted by any of the ranchers.

Sheep.—Only limited numbers of sheep have been treated with ET-57, but no symptoms of poisoning have been noted in sheep given doses of 100 mg./kg. or less. One yearling ewe given 400 mg./kg. showed a mild diarrhea, but nothing more. Oscillographic display of the

[1] Accepted for publication November 11, 1956.

[2] Sampled as Dow ET-14.

Table 1.—Summary of results of dosing cattle with Dow ET-57.

Animal	Dose (Mg./Kg.)	Number Treated	Number Affected			Number Unaffected	Symptoms
			Severely	Moderately	Mildly		
Mature cows	250	8	3	3	2		Depression, diarrhea, inappetence, salivation, dyspnea
Yearlings	400	4	4				Severe weight loss, depression, diarrhea, inappetence, affected over a 3-week period
	200	6	2	4			Moderate depression, diarrhea, some salivation
	150	7		3		4	Stiffness, diarrhea, some salivation
	125	6			1	5	Weakness, mild depression of a few hours' duration
	100	6				6	None
Calves, 4 months of age	150	2				2	None
	100	3			1	2	Very mild
Dairy calves, 1 to 2 weeks of age	100	4				4	None

Table 2.—Cholinesterase values on cattle given oral doses of Dow ET-57.

Dose (Mg./Kg.)	No. Treated	Erythrocyte Cholinesterase, Expressed in 0.01's Δ pH Units/Hr.							
			Time After Dosing						
				Weeks					
		Control	1 Day	1	2	3	5	7	15
				Yearling and Mature Cows					
400	4	60[a]	26	6	6	9	17	19	54
		40–95	22–38	5–8	4–8	3–15	6–29	11–32	45–69
250	8	48	36(4)[b]	17(6)	17(7)		21(6)	23(3)	
		36–68	14–47	5–34	11–24		9–30	18–28	
200	6	48	34	15	15	22	34	23(3)	50(2)
		36–63	15–47	10–21	9–20	14–34	15–49	21–24	48–51
150	7	55	48	22	28	32	27	30(3)	50(2)
		48–64	38–62	16–29	19–38	22–45	22–39	29–30	49–52
125	6	47	37	39	48	33	34	43	41(4)
		37–66	23–46	21–52	23–57	19–41	28–44	26–63	38–68
100	6	64	53	45	30	32	52	45(1)	
		56–75	44–61	24–59	23–35	25–36	28–82		
						Calves			
150	2	56	39	32	46	44	32	30	
		55–57	36–43	27–38	44–47	37–51	25–39	28–32	
100	7	46	30	22	26	39(3)	35(3)	34(3)	56(3)
		37–53	14–36	17–39	18–39	36–45	27–41	29–41	47–61

[a] Upper figure is average for group; lower figures, the range.
[b] Number in parentheses indicates number of animals examined at that time, others having been removed from the test.

heart action of this sheep over a period of 3 days failed to reveal any effects of this dosage.

Symptoms.—Dow ET-57 has been usually considered as an organic phosphorus insecticide, but it failed to act predominantly as such. The predominant symptoms were those associated with phenolic compounds. The depression of cholinesterase was also different from most organic phosphorus compounds in that the depression took place over a period of days, usually reaching the lowest point in from 6 to 8 days after dosing. Recovery of cholinesterase was slow, averaging 1% or less per day. (See table 2.)

Dow ET-57 appeared to have at least two modes of action, possibly representing the stages of its detoxification. The initial symptoms were essentially those considered typical for chlorinated phenols and included depression, muscular weakness, incoordination, and prostration. Diarrhea usually occurred. These symptoms appeared during the first 24 hours and might increase in intensity with time, depending upon the amount of the compound given. At the higher dosages the weakness persisted for several weeks. The diarrhea was usually abated in 4 to 5 days. At low dosages the chlorinated phenol syndrome was all that was seen. At higher dosages a second, less defined, syndrome appeared, in which salivation and dyspnea were superimposed upon the initial syndrome. At the higher dosages the animals lost weight rapidly, the hair lost luster, and they presented a general appearance of unthriftiness.

Recovery time depended upon the severity of the affliction and in turn upon the dosage administered. Some of our cattle treated with 400 mg./kg. had not returned to normalcy after 100 days.

In this series of studies we did not kill cattle with Dow ET-57. The highest dosage (400 mg./kg.) employed represented four times the dosage to be employed in cattle treatment, and, since there was no other source of exposure, it would be unlikely that cattle would ever consume this amount intentionally or accidentally.

SUMMARY AND CONCLUSIONS.—Dow ET-57 was administered orally to 254 cattle at various dosages. Mild symptoms of poisoning were observed at 125 mg./kg. in cattle, and progressively more severe symptoms as the dosage was increased to 400 mg./kg.

One sheep showed a mild diarrhea at 400 mg./kg.

Dow ET-57 at first produced symptoms similar to chlorinated phenols; then of organic phosphorus insecticides. First, there was muscular weakness, incoordination prostration, and diarrhea, occasionally salivation and dyspnea, the whole accompanied by severe weight loss at the higher dosages.

Cholinesterase was depressed progressively over a period of 6 to 8 days. Recovery proceeded at the rate of approximately 1% per day.

No deaths occurred at any of the dosages employed. There is evidently a wide range between the minimum toxic and minimum lethal dosage.

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Effects of BHC with High and Low Gamma Isomer Content and of Lindane upon the Cotton Aphid[1]

ROBERT H. MOUNT and F. S. ARANT, *Agricultural Experiment Station, Alabama Polytechnic Institute, Auburn*

The cotton aphid, *Aphis gossypii* Glover, is an important pest on cotton throughout the cotton belt. From 1947 until 1953 BHC dusts containing 3 per cent gamma isomer were highly effective in control of this insect (Arant 1947, 1950; Gaines & Young 1948; Young & Gaines 1950). In 1953, however, Arant reported that the cotton aphid was occurring in damaging numbers in Alabama in cotton fields being treated with 3–5 BHC-DDT dusts. Young & Gaines (1953) noted that a 3-5-40 BHC-DDT-sulphur mixture alternated with calcium arsenate failed to prevent an increase in aphid numbers on cotton plots when these materials were applied at standard rates.

Several theories have been advanced to explain these and other recent poor performances of BHC against the cotton aphid. Most of these theories place the blame either on resistance or on the source materials used to prepare the 3 per cent gamma isomer dusts. Proponents of the latter view would explain the decreased effectiveness of BHC against the aphid with a recent trend away from the use of low gamma isomer source materials to source materials of higher gamma isomer content. This trend has, in effect, resulted in a final product containing substantially less of the non-gamma isomers of BHC than the earlier formulations.

Field and laboratory experiments were conducted in Alabama in 1954 and 1955 to investigate some of the factors that may exert an influence on the toxicity of BHC to the cotton aphid.

PROCEDURE.—*Field Experiments.*—Field experiments were conducted at Prattville (1954) and at Prattmont

[1] These data are taken from a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. Degree in Entomology at the Alabama Polytechnic Institute. Accepted for publication November 8, 1956.

(1955) to determine the effects of high and low gamma isomer content of BHC and of lindane upon the cotton aphid and to determine the effects of DDT and methods of dilution upon the toxicity of BHC dusts to the aphid. These two localities were about 6 miles apart. The treatments in each of these experiments were as follows: (1) BHC with low gamma isomer content (ground), (2) the same BHC (ground) plus 5% DDT, (3) BHC with high gamma isomer content (ground), (4) the same BHC (ground) plus 5% DDT, (5) the same BHC (impregnated), (6) the same BHC (impregnated) plus 5% DDT, (7) lindane, (8) lindane plus 5% DDT, and (9) an untreated check.

The insecticides were applied at 3% gamma isomer concentrations with a rotary-type hand-duster at the rate of approximately 20 pounds per acre. The BHC with high and low gamma isomer content and the lindane dusts were prepared from technical materials containing 40.0, 15.1, and 100.0% of the gamma isomer, respectively.

For the first experiment (Prattville, 1954), a cotton field situated on flat, uniform terrain was selected. The cotton was treated six times at 5-day intervals with calcium arsenate to induce the development of a high population of the cotton aphid. On August 10 the population had reached a suitable level, and the dusts were applied in late afternoon on that date. Each treatment was replicated four times on plots randomized in complete blocks. The plots were 70 feet in length and five rows in width and were separated by two rows of untreated cotton.

Aphid populations were determined on each plot by counting the number of live aphids on a square inch of the undersurface of each of 20 leaves. In each instance, the fourth leaf from the terminal was selected from plants chosen at random from the middle row of the plot. Counts were made on each of the 2 days prior to application and at 24-, 48-, and 96-hour intervals after application. Yield records were taken at time of harvest.

The second field experiment (Prattmont, 1955) was conducted in much the same manner as that of the previous year. Calcium arsenate was applied to the field five times between July 2 and 21. The plots consisted of four 50-foot rows each and were separated by three rows of untreated cotton. The dusts were applied on the morning of August 2 and on the afternoon of August 4. Aphid population counts were made immediately before and at 24- and 48-hour intervals after the first application and at 24-, 48-, 96-, and 168-hour intervals after the second. Yield records were taken.

At the conclusion of this experiment samples of the BHC with low gamma isomer content (ground), with high gamma isomer content (ground), with high gamma isomer content (impregnated), and of the lindane were sent to the State Chemical Laboratory for analysis on the basis of gamma isomer content.

Laboratory Experiment.—A laboratory experiment was conducted at Auburn to determine whether differences in toxicity to the cotton aphid existed among sprays containing BHC with high and low gamma isomer content and lindane. Aphids used in this investigation were of a local strain and were cultured on cotton plants grown in the greenhouse.

Emulsifiable concentrates containing 2.5 mg. of gamma isomer per ml. were prepared from each of the following technical materials: BHC (15.0% gamma isomer), BHC (41.0% gamma isomer), and lindane (100% gamma isomer). Acetone was used as a solvent to which was added 1.0% of an emulsifier (Atlox 1045 AB-849 C).

Preliminary tests designed to arrive at a rough estimate of the toxicities of the chemicals to the aphid were then conducted. Infested leaves were sprayed in a small spray-tower with emulsions of BHC with high and low gamma isomer content and lindane at each of the following dosage-levels: 0.01, 0.5, 1.0, and 1.5 mg. of gamma isomer per ml. Only one leaf was used per treatment. The leaf was placed lower-side-up in a petri dish in the base of the tower and sprayed for a period of 10 seconds at a pressure of 20 p.s.i. The leaf was left in the tower for 1 minute longer to allow the mist to settle. The surface of the leaf and the aphids present were throughly wetted, but not to the extent that the liquid pooled. The sprayed leaf was removed from the tower, placed on a piece of broadcloth that had been stretched over an embroidery hoop, and covered with a petri dish. When all of the leaves had received their respective treatments, they were transferred to a room in which the temperature was maintained at 80° F. At the end of 24 hours, the living and dead aphids were counted.

Preliminary tests indicated that the LD-50 concentrations of the formulations to the aphids were between 0.01 and 0.20 mg. of gamma isomer per ml. Hence, infested leaves were sprayed with each of the three formulations at the following dosage levels: 0.01, 0.05, 0.10, 0.15, and 0.20 mg. of gamma isomer per ml. A check was included, consisting of a leaf sprayed with a concentration of solvent plus emulsifier equivalent to that contained in the most concentrated sprays. The experiment was replicated four times. One replicate was performed on each of the following dates: December 15 and 16, 1955, and January 5 and 7, 1956. Before determining percentage of mortality in the experiment, the aphids under each dish were grouped according to size as first instar nymphs, intermediate nymphs, or adults.

RESULTS AND DISCUSSION.—*Field Experiments.*—The results of the first experiment (Prattville, 1954), based on average numbers of the aphid per square inch following insecticidal treatments, are presented in table 1.

The aphid was controlled effectively with dusts containing 3% gamma isomer applied at the approximate rate of 20 pounds per acre regardless of source material, method of formulation, or presence or absence of DDT in the mixture. Aphid numbers following treatment were significantly lower on all dusted plots than on the checks.

There was no consistent evidence that dusts prepared from BHC with low gamma isomer content differed in effectiveness from those prepared from BHC with high gamma isomer content or lindane. The only differences among insecticides that appeared significant were in the infestation counts made on August 14, 96 hours after treatment. Some differences in this count were significant. However, the involvement of extremely small numbers of the aphid on the treated plots on that date would seem to render any numerical differences meaningless.

Following treatment there was a great decline in the numbers of aphids on the checks (table 1). Most of this decline was attributed to insecticidal drift. Applications

Table 1.—Average infestations of cotton aphid on plots treated with 3% dusts made from BHC with high and low gamma isomer content and from lindane, Prattville, 1954.

Treatment[a]	Dates and Average Number Aphids per Square Unch			
	Aug. 10	Aug. 11	Aug. 12	Aug. 14
Check	17.8	6.4	4.4	6.0
High gamma BHC (gr.) +5% DDT	11.3	0.7	0.5	0.9
Low gamma BHC (gr.)	12.1	1.1	1.1	1.2
High gamma BHC (imp.)	12.9	1.9	1.3	1.6
Low gamma BHC (gr.) +5% DDT	12.9	2.4	1.2	1.3
Lindane	13.6	0.8	0.5	0.9
High gamma BHC (gr.)	11.5	1.4	0.4	0.9
Lindane+5% DDT	11.0	1.1	0.6	0.8
High gamma BHC (imp.) +5% DDT	14.4	1.5	0.8	0.9
L.S.D. @ 5% level	—	3.4	1.9	0.4

[a] Dusts applied after counts were made on August 10. Four replicates.

were made in late afternoon, and atmospheric conditions at that time were such that the insecticides hovered near the ground and dispersed slowly over the entire area. Populations of predators and parasites were somewhat low during the experimental period and seemed to have had little effect upon the aphid numbers.

There were no significant differences in yields resulting from the various treatments.

In the second field experiment (Prattmont, 1955), none of the treatments resulted in satisfactory control of the aphid (table 2). However, the aphid infestations on most of the treated plots were significantly lower than those on the checks 24 hours after the first application of the insecticides. Following the second application, there were no significant reductions in aphid populations by any of the insecticidal treatments when compared with the untreated checks. By August 11, 9 days after the experiment was begun, the aphid population had declined sharply on all plots as a result of the activity of beneficial insects and other factors. At this time there were significantly fewer aphids on the check plots than on most of the treated plots.

The relative ineffectiveness of the insecticides against the aphid in this experiment made it difficult to evaluate differences due to source materials used. Twenty-four hours after the first application a dust prepared from BHC (ground), with low gamma isomer content, and DDT had reduced the aphid infestation to a significantly lower level than the comparable BHC formulation with high gamma isomer content. Also, the dusts with low gamma isomer content appeared to be slightly, but not significantly, more effective than the one with high gamma isomer content (impregnated). However, the dusts with high and low gamma isomer content (ground) that did not contain DDT were equally effective in reducing aphid populations. The lindane formulations appeared to be somewhat more effective than any of the other insecticides. However, subsequent chemical analyses of the dusts revealed that the lindane contained slightly higher concentrations of the gamma isomer than the other samples of formulations analyzed for this purpose.[2]

There were no significant differences between yields from treated and untreated plots, nor among any of the treated.

A study of the data from this experiment indicated that the aphids present in this field had developed some degree of resistance to BHC prior to the time of this experiment.

Laboratory Experiments.—In the laboratory, preliminary investigations revealed that the younger aphids were more susceptible to the effects of BHC than the older individuals. Thus, the aphids were grouped according to size, and the following results are presented on that basis.

All formulations of BHC and lindane were highly effective in killing the aphids. Analysis of variance revealed no significant differences in toxicity among the three materials. Adults were omitted from the statistical analysis owing to their absence on many of the treated leaves. All formulations were significantly more toxic to the first instar nymphs than to the intermediate forms.

As there were no significant differences among the formulations with regard to toxicity, it was possible to construct dosage-mortality curves using the averages of mortality figures obtained with all insecticides at given dosage levels. In constructing these curves, percentages of mortality were converted to angles before plotting against concentrations of the gamma isomer. Figure 1 shows the curves for each of the aphid groups. The LD-50 concentrations of BHC to the cotton aphid adult, inter-

[2] Gamma isomer content of the dusts expressed in percentages were as follows: lindane, 3.28; low gamma (ground), 2.95; high gamma (ground), 2.90; and high gamma (impregnated), 2.99

Table 2.—Average infestations of cotton aphid on plots treated with 3% dusts made from BHC with high and low gamma isomer content BHC and from lindane, Prattmont, 1955.

Treatment[a]	Dates and Average Number of Aphids per Square Inch						
	Aug. 2	Aug. 3	Aug. 4	Aug. 5	Aug. 6	Aug. 8	Aug. 11
Check	26.8	18.7	16.2	10.1	7.1	4.1	0.4
High gamma BHC (gr.)+5% DDT	19.8	17.6	11.5	4.3	4.9	4.6	2.5
Low gamma BHC (gr.)	24.8	13.8	10.9	7.4	7.0	5.8	0.4
High gamma BHC (imp.)	24.7	16.1	12.0	5.5	6.5	4.5	1.9
Low gamma BHC (gr.)+5% DDT	22.1	12.6	10.9	6.6	6.4	6.3	2.9
Lindane	24.7	11.0	9.1	4.5	3.9	4.7	1.2
High gamma BHC (gr.)	22.8	14.6	11.6	6.9	5.9	5.1	2.4
Lindane+5% DDT	19.0	14.9	12.9	7.4	6.7	4.6	2.3
High gamma BHC (imp.)+5% DDT	22.0	13.9	10.9	5.0	5.2	5.4	1.9
L.S.D. @ 5% level	—	3.9	n.s.	n.s.	n.s.	n.s.	0.9

[a] Insecticides applied on August 2 and 4. Four replicates.

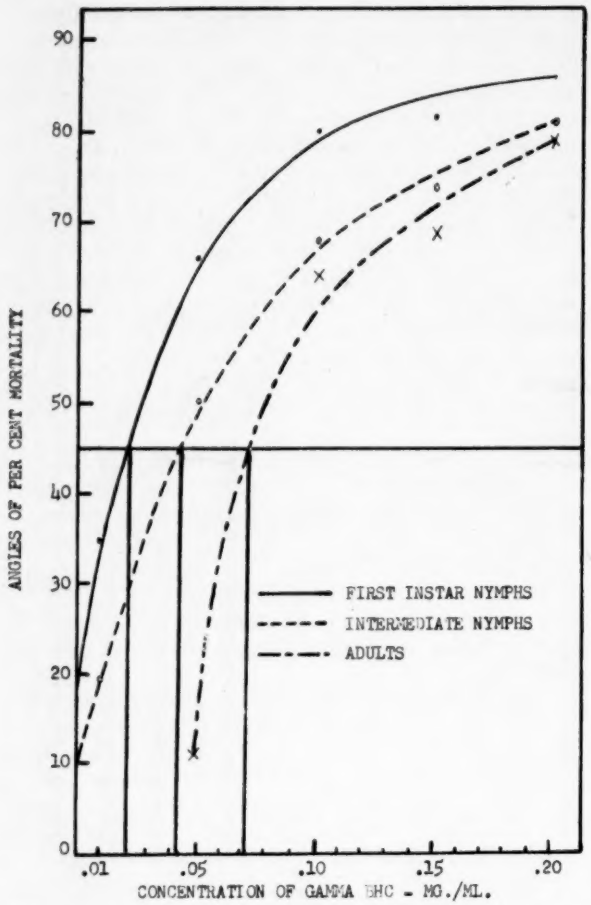

FIG. 1.—Dosage-mortality relationships between gamma isomer of BHC and three stages of the cotton aphid, Auburn, Alabama. 1955.

mediate nymph, and first instar nymph were determined to be 0.072, 0.044, and 0.021 milligrams of gamma per milliliter, respectively.

INTERRELATIONSHIPS OF EXPERIMENTS.—There was a wide diversity between the degrees of cotton aphid control obtained with the BHC dusts in the two field experiments, although the same methods and materials were applied in both cases, and the same variety of cotton was used. In the experiment conducted in 1954, one dust application resulted in effective control of the aphid, whereas two applications failed to control the insect in the 1955 experiment. There were several factors extrinsic to the aphid that could have been partially responsible for this difference. It is possible that atmospheric conditions at the time of application of the insecticide were more conducive to high aphid mortality in the former experiment than in the latter. Also, the vigor of the cotton plants during the experimental period could have been a factor, as ecological conditions in 1955 were more favorable for the growth of cotton than in 1954. This was particularly true with regard to availability of water early in the season. However, it is extremely doubtful that these factors alone were responsible for the much greater tolerance to BHC exhibited by the aphid in 1955. It should be noted here that the experimental period, in both instances, was characterized by hot, dry weather, and that in each case the cotton plants were almost mature.

The two sets of data were in agreement on one basic point, namely: that there were no inherent differences among the insecticides with regard to their effectiveness against the aphid. The laboratory results closely paralleled those of the field experiments regarding differences due to source materials used. Thus, it was concluded that none of the factors that were investigated involving source materials used, method of preparation, and addition of DDT to the BHC dust (3% gamma isomer) was associated with the decreased effectiveness of the product against the aphid.

Although most of the inferences derived from this study indicate that resistance was playing an important role in the problem of cotton aphid control with BHC, it would be presumptuous to draw a definite conclusion until confirmed by further research.

SUMMARY.—Two field experiments and a laboratory study were conducted in Alabama in 1954 and 1955 in which various formulations prepared from BHC with high (40%) and low (15.1%) gamma isomer content and from lindane were tested against the cotton aphid.

The results of the field experiments revealed no distinct differences among insecticides used with respect to aphid control. However, a wide variation in degree of control did occur between two experiments. In the first experiment (Prattville, 1954), effective control of the aphid was obtained with one application of each formulation of dust containing 3% gamma isomer whereas in the second (Prattmont, 1955), aphids remained numerous after two applications of dust.

The results of the laboratory study generally paralleled those of the first field experiment. All gamma isomer formulations were highly effective as sprays in killing the aphid. No significant differences were observed among the toxicities of the insecticides to the insect. It was found, however, that the first-instar nymphs were significantly more susceptible than the intermediate nymphs. Dosage-mortality curves of gamma isomer to the adult aphids and to first-instar and intermediate nymphs showed the LD-50 concentrations to be 0.072, 0.021, and 0.044 mg. of gamma isomer per ml., respectively.

None of the factors that were investigated involving source materials used, methods of manufacturing and processing the 3% dust, and the addition of DDT to the final product was associated with the decrease in effectiveness of BHC against the aphid. Although there were definite indications that resistance was a factor, further research is needed before conclusions to that effect can be drawn.

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Further Tests of Band Placement of Insecticides for Clover Root Borer Control[1]

C. R. WEAVER, K. P. PRUESS and J. L. PARSONS,[2] *Ohio Agricultural Experiment Station, Wooster*

Weaver & Haynes (1955) described a method of placement of insecticides in bands under red clover seed that achieved control of the clover root borer, *Hylastinus obscurus* (Marsh.). Band placement was as effective at 0.75 pound of toxicant per acre as broadcast treatments at 1 pound or more as reported by Gyrisco *et al.* (1954) and App & Everly (1950).

Since the 1951–53 tests were carried out in one location on small plots and applied by a farm crew experienced in experimental procedures, it was desired to test the band placement method more extensively. Tests in 1954–56 were conducted at seven different locations, using different band seeding equipment operated by farmers as well as technically trained personnel. The results of these trials are reported in this paper.

BAND SEEDING TESTS.—In 1954 at Wooster, Ohio, in two separate tests, insecticides at different rates were seeded with oats. The same basic technique of planting as that described by Weaver & Haynes (1955) was employed. Each treatment was replicated three times with individual plots measuring 24×45 feet.

Aldrin at three rates and heptachlor at two rates were compared in one test. Either insecticide at 0.75 pound of toxicant per acre gave satisfactory root borer control and increased yield. Results are tabulated in table 1.

Lindane at three rates was compared with aldrin at a single rate in the second test. All rates and each insecticide gave good control of the root borer. Yield values were erratic because of an infestation of ladino clover in some of the plots. Nonsignificant differences were therefore recorded. At both the second and third cuttings, however, all the treatments resulted in more yield than the check (Table 2).

Table 1.—Clover root borer control with various rates of aldrin and heptachlor in band seedings of red clover. Wooster, Ohio. 1955.

INSECTICIDE	POUNDS PER ACRE	PER CENT CONTROL[a]	YIELD TONS/ACRE
Aldrin	0.25	30.0	1.88
	.75	87.1	1.88
	1.50	92.8	1.94
Heptachlor	.25	57.1	1.73
	.75	98.7	1.97
Check			1.67
L.S.D. at 5% level			0.19

[a] Based on borers in 30 roots per treatment. The untreated check had 2.33 borers per root.

During the period 1954–56 nine additional band placement tests were observed. In 1954–55 insecticides in band seeding were applied to two red clover variety trials in Wood and Mahoning Counties. In these tests duplicate blocks of several varieties received 0.75 pound of aldrin in the fertilizer while other duplicate blocks were band seeded without insecticide. In four other tests observed in 1954–55, one- to three-acre portions of single fields were band seeded with insecticide while the remainder of the field served as a check.

Three variety trials were conducted in 1955–56, one each in Wayne, Wood and Mahoning Counties. Again, duplicate blocks of several varieties were treated with two other blocks serving as checks. Root borer populations in these nine trials are given in table 3.

Considering only the 0.75 pound toxicant rate of aldrin, heptachlor or lindane, the band placement method in these 11 tests gave an average of 89.3% control of borers, the range of control varying from 70.7 to 100%. In every case but one where yields of second and third cutting were measured the treated plots yielded more than the check plots. The average increase in second cutting yields in seven trials was 430 pounds of dry weight of hay per acre.

From these trials one can conclude that band seeding of red clover with 0.75 pound of insecticide will result in satisfactory control of the clover root borer with a resulting increase in yield of hay.

The band seeding method described has been employed with most success in legume seedings with oats or summer seedings without a companion crop. Band seeding has not been as successfully adapted for seeding in wheat. Therefore, some other method of applying a seed-insecticide combination needs to be developed.

Surface Application.—In March 1955, red clover seed was mixed with a granulated formulation of aldrin (10% on Attaclay AA, 30 to 60 mesh) and sown in wheat in a randomized block design with four replications of 30×35 foot plots. The seed tubes of the drill were extended so that they came close to the ground and placed the seed-insecticide mixture in bands along the ground. Propor-

[1] Accepted for publication November 16, 1956.

[2] Assistant Professor in Entomology, Research Assistant in Entomology, and Assistant Professor in Agronomy, respectively.

Table 2.—Clover root borer control with aldrin and lindane in band seedings of red clover. Wooster, Ohio. 1955.

Insecticide	Pounds per Acre	Per Cent Control[a]	Yield (Tons/Acre) 2nd Cutting	Yield (Tons/Acre) 3rd Cutting
Aldrin	0.75	85.6	2.01	0.71
Lindane	.75	78.5	1.95	.72
	1.25	85.6	1.88	.67
	1.75	88.6	1.88	.71
Check			1.80	.58

[a] Based on borers in 30 roots per treatment. Untreated check had 2.33 borers per root.

tions of seed and clay were regulated so that a dosage of 0.0, 0.75 and 1.5 pounds of actual aldrin was delivered per acre. In August 1956, the plots were sampled by taking a total of 40 roots from the check and 80 roots from each treatment. The borers per root averaged 1.48 in the check, 0.35 in the 0.75-pound treated areas and 0.06 in the 1.5-pound treated areas. The method was successful enough to warrant further testing under a variety of conditions.

The land upon which the 1952–53 trials were conducted was carried through the usual corn, small grain, hay rotation. In 1955, red clover was seeded in oats without further application of insecticide. In 1956, the root borer populations were recorded. The average borers per root in the various treatments was 3.31, in the checks 3.67. Apparently little carry-over effect of the insecticides remained 4 years after their application. For continuing root borer control the band application of insecticide would require renewal in each rotation.

SUMMARY.—Field trials were conducted in several locations in Ohio to establish the effectiveness of band placement of insecticides for control of the clover root borer. Aldrin was used most extensively, although lindane and heptachlor were also included. In 13 trials 0.75 pound per acre of one of the three insecticides gave an average of 89.3% control. In seven trials in which yields of hay were taken the insecticide treated plots yielded an average of 430 pounds per acre more than the untreated plots.

In a single test, insecticide placed on the surface of the soil with the seed at the normal seeding time in wheat gave good control of the clover root borer.

Residual action of materials placed in the soil was not sufficient to control borers in a planting made 4 years later.

Table 3.—Clover root borer control with band placement of 0.75 pound of aldrin per acre in several locations in Ohio. 1955–56.

County	Roots per Treatment	Borers per Root in Aldrin	Borers per Root in No Aldrin	Per Cent Control	Yield[a] (Tons/Acre) Aldrin	Yield[a] (Tons/Acre) No Aldrin
		1955				
Wood	100	0.25	1.28	80.5	1.96[b]	1.38
Mahoning	100	.35	2.59	86.5		
Van Wert 1	30	.10	5.27	98.1		
Van Wert 2	30	.87	2.97	70.7		
Van Wert 3	30	.00	2.27	100.0		
Trumbull	30	.06	4.70	98.7		
		1956				
Wayne	120	.02	1.94	98.8	1.01[c]	0.95
Wood	100	.09	0.79	88.6	0.5[b]	0.5
Mahoning	100	.11	1.00	89.0		

[a] Second cutting.
[b] L.S.D. at 1% level = 0.14.
[c] Not significant.

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Comparative Toxicity Tests as an Aid in Selecting Insecticides for Control of the Cherry Fruit Fly[1]

KENNETH E. FRICK,[2] *Irrigation Experiment Station, Prosser, Washington*

The cherry fruit fly, *Rhagoletis cingulata* (Loew), is by far the most important pest of cherries in the Yakima Valley of Washington. Studies on its control have been directed primarily towards more suitable insecticides since about two-thirds of the crop is packed for the fresh fruit market. The "ideal" insecticide for pre-harvest control of the cherry fruit fly in the arid eastern Washington cherry districts should have the following characteristics: (1) leave no visible residues on the fruit, (2) have a residual action of 10 to 14 days, (3) be of low mammalian toxicity, (4) be toxic to San Jose scale crawlers, spider mites, black cherry aphid, shot hole borer, and pear-slug.

To facilitate the testing and screening of promising materials, a Hoskins-Caldwell spray chamber (Hoskins & Caldwell 1947) was built. The specifications described by Caldwell (1948) were incorporated into the chamber. With this chamber, direct comparisons can be made of the relative value of insecticides as contact sprays. After these studies the most promising materials, considering also the reported residual value and mammalian toxicity of each, are included in the orchard tests. Several insecticides, known to have no residual value, were tested because such materials are needed to knock down the flies during the harvest period.

MATERIALS AND METHODS.—1. *Obtaining the Adults.*—Fortunately it is not difficult to have a continuous source

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[2] The writer is grateful to Roy E. Bry for conducting most of the tests and to Carl A. Johansen for designing the containers for adult emergence.

of adults for the tests. During cherry harvest, heavily infested fruits are picked and piled upon $\frac{1}{4}$" mesh hardware cloth held a few inches above the ground by frames placed under the trees. Under each frame unbleached muslin is laid down to prevent the emerging larvae from entering the ground. About 1 inch of soil is spread smoothly on the cloth. This gives the larvae a place to pupate that is shaded, kept humid by the ground below, and that can be quickly sifted.

During sifting the puparia are held, together with some soil and litter, in a 16-mesh screen. This material is placed in quart ice cream cartons and placed in refrigeration at 35° to 40° F. There have been as many as 3,000 puparia per quart but the average has been around 1,500. Frick *et al.* (1954) showed that a minimum of 150 days in refrigeration will produce maximum emergence. After the diapause is broken, the pupal material is removed at the rate of 1 quart every 3 or 4 days and held at 80° F. About 24 days are required to produce the first fly emergence. The peak of emergence occurs about 2 weeks later. The total emergence period lasts about 1 month.

The emergence containers are of sheet metal and consist of two parts. The bottom is 8" in diameter and 4" high, and can accommodate 2 quarts of puparia and litter. The top slips snugly down over the bottom, is 10" high, and covered on top with 16-mesh screen. After the flies have hardened and started to show their full coloration, they have a positive phototaxis and move to the top of the container. Therefore adults from about 1 to 24 hours of age are obtained by removal of the top each 24 hours. Each top is set on an ordinary pie pan and placed in a freezer. As soon as the flies fall to the bottom, those in each pan are poured into one pan to give a single population. The flies are then tapped into the spray chamber cages in about equal numbers.

2. *Spray Chamber Operation.*—Johansen *et al.* (1954) gave the results of testing methoxychlor and parathion in the spray chamber. In the present studies six materials were tested, using the glass atomizer described by Johnasen *et al.* A metal atomizer that atomized between 5.1 and 4.6 ml. of liquid in 40 seconds at an air pressure of 8.7 p.s.i. (450 mm. mercury) was constructed. From 75 to 77% of the droplets that fell on slides coated with vaseline measured between 7.5 and 59 microns in diameter. Johansen *et al.* reported an average droplet diameter of 56 microns for the glass atomizer.

Usually two insecticides were tested each day. A thorough cleaning and $\frac{1}{2}$-hour wait was allowed between tests. The check cage was always first in a series, and distilled water or 1% xylene emulsion was used for the spray. The lowest concentration of insecticide followed the check. Each subsequent cage received the next higher concentration. Each cage was left in the chamber for 10 minutes after spraying.

An average of 591 flies, divided into three tests, were used during the time that the glass atomizer was employed. With the metal atomizer, the totals averaged 831 flies in five replications.

3. *Post-Treatment Care of Flies.*—All cages were held at room temperatures until the last one was out of the chamber. Then the five cages, including check, were placed in a temperature cabinet of 27 cu. ft. volume, 70° F., at relative humidities of 70 to 80%. Seventy degrees was chosen because that temperature is closer to preharvest orchard temperatures than the 75° F. used by Johansen *et al.* No food or water was provided at 70° F. as mortalities in the check were low, averaging 3.7% and varying from 0 to 6.9%. Counts were made after 24 hours. Badly affected and moribund flies were considered dead.

Table 1.—Comparative toxicities of several insecticides to the cherry fruit fly, using a glass atomizer.

Insecticide	LD_{50}	Fiducial Limits 5% Level	Toxicity Index	LD_{95}	Fiducial Limits 5% Level	Toxicity Index
Parathion	0.039	±0.005	100	0.158	±0.047	100
Demeton	.048	± .006	81	.152	± .040	123
EPN	.061	± .007	64	.323	± .083	49
Perthane	.166	± .017	23	.644	± .125	24
Dilan	.291	± .035	13	.883	± .145	18
Malathion	.497	± .042	8	1.291	± .178	12

4. *Insecticides and Formulations.*—All insecticides were in the emulsifiable form. The concentrations used were based upon the percentage of actual insecticide in each formulation. Two drops of *Triton X*-100[3] were included in each 100 ml. of stock solution to aid emulsification. The insecticides included in the tests, the percentage of active ingredient of each, and the companies supplying them are given below:

parathion, 25%, American Cyanamid Co.
malathion, 57%, American Cyanamid Co.
Am. Cyanamid 4124, 25%, American Cyanamid Co.
Diazinon, 25%, Geigy Agricultural Chemicals.
Pirazinon, 25%, Geigy Agricultural Chemicals.
demeton, 23%, Pittsburg Agricultural Chemical Co.
EPN, 45%, E. I. duPont de Nemours and Co.
DDVP technical, 100%, Montrose Chemical Co.
TEPP, 40% tetraethyl pyrophosphate, 60% other ethyl phosphates, Niagara Chemical Div.
Dipterex technical, 100%, Chemagro Corp.
Phosdrin (Shell OS 2046) technical, 100%, Shell Development Corp.
aldrin, 23.1%, Shell Development Corp.
heptachlor, 23%, Velsicol Chemical Corp.
chlordane, 72%, Stauffer Chemical Co.
Dilan, 25%, Commercial Solvents Corp.
Perthane, 25%, Rohm and Haas Co.
Strobane, 72%, Goodrich Chemical Co.
methoxychlor, 25%, Geigy Agricultural Chemicals (designated G in table 2).
methoxychlor, 24%, E. I. duPont de Nemours and Co.

Results and Discussion.—The data obtained from the tests were subjected to the probit analysis method (Finney 1952). The LD_{50} and LD_{95} with the fiducial limits of each are given for each insecticide in tables 1 and 2. The relationships between the insecticides tested with the glass atomizer are shown in figure 1 in the form of regression lines. Figure 2 gives the regression lines of selected insecticides that had been tested with the metal atomizer.

The toxicity index developed by Sun (1950) was employed for the direct comparison of insecticides regardless of which atomizer had been used. Parathion was chosen for the standard material and therefore has a value of 100 (table 1 and 2). This index

$$\left(\text{Toxicity index} = \frac{LD_{50}\ (\text{or}_{95})\ \text{of the standard (parathion)}}{LD_{50}\ (\text{or}_{95})\ \text{of a test insecticide}} \times 100\right)$$

provides a quick means by which to compare materials

[3] Manufactured by Rohm & Haas Co.

Table 2.—Comparative toxicities of several insecticides to the cherry fruit fly, using a metal atomizer.

Insecticide	LD_{50}	Fiducial Limits 5% Level	Toxicity Index	LD_{95}	Fiducial Limits 5% Level	Toxicity Index
DDVP	0.006	±0.002	271	0.009	±0.001	702
Diazinon	.010	± .001	157	.041	± .001	161
Pirazinon	.011	± .001	152	.048	± .008	137
Aldrin	.013	± .002	123	.075	± .020	88
Phosdrin	.014	± .001	114	.050	± .011	132
Parathion	.016	± .002	100	.066	± .018	100
TEPP	.021	± .002	76	.067	± .014	98
Demeton	.022	± .001	73	.050	± .010	132
Heptachlor	.022	± .002	73	.061	± .012	108
Am. Cyanamid 4124	.023	± .002	70	.079	± .012	83
Dipterex	.044	± .005	36	.234	± .067	28
Perthane	.077	± .005	21	.185	± .021	36
Malathion	.104	± .013	15	.560	± .133	12
Chlordane	.106	± .009	15	.327	± .064	20
Strobane	.159	± .035	10	.895	± .209	7
Methoxychlor, G	.285	± .047	5.6	1.142	± .351	5.8
Methoxychlor, dP	.289	± .034	5.5	1.061	± .348	6.2

that had been tested under different conditions. The tables show that, at the LD_{50} index, demeton, Perthane and malathion give reasonably consistent indices although malathion was one of the more erratic materials used. The relatively high fiducial limits show how erratic malathion is, and in another series of tests employing the metal atomizer, malathion gave an LD_{50} of 0.145.

Sun's index gives values above 100 for materials that are superior to the test insecticide. Thus DDVP has an LD_{95} of about one-seventh that of parathion and therefore has a toxicity index about seven times greater than the index of parathion (table 2). When the index is below 100, more of the insecticide is required to give a kill equal to that of parathion. For example, chlordane has an LD_{95} five times as great as parathion and an index one-fifth that of parathion.

There are a few insecticides such as demeton, DDVP, TEPP, and heptachlor that have relatively higher LD_{95} indices in proportion to the LD_{50}'s than parathion (tables 1 and 2). This fact is shown in the figures by the steepness of the lines. Such a characteristic, providing an increasingly greater kill with each rise in concentration, is an advantage in fruit fly control. In a short-season fruit that is shipped fresh, as are cherries, the maximum kill is desired with a dosage so low that minimum toxic residues remain on the fruits at harvest time.

Certain other materials exhibit just the opposite result.

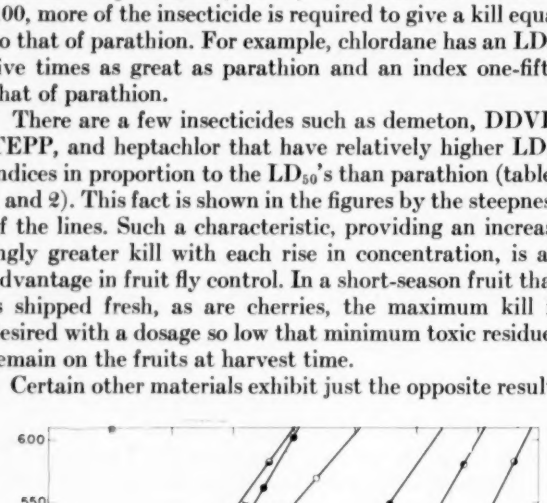

FIG. 1.—Relationships between probit of kill and dosages of several insecticides using the glass atomizer.

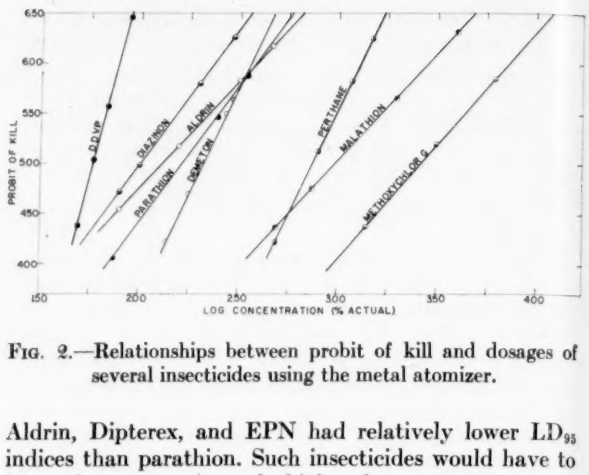

FIG. 2.—Relationships between probit of kill and dosages of several insecticides using the metal atomizer.

Aldrin, Dipterex, and EPN had relatively lower LD_{95} indices than parathion. Such insecticides would have to be used at proportionately higher dosages to give a satisfactory kill of the adults. For this reason, these materials are not considered among the more promising.

Several insecticides proved to be superior to parathion as contact sprays at both the LD_{50} and LD_{95} index levels. DDVP was extremely toxic to the flies but lacks residual value. Its value in practical control would be limited to close-to-harvest treatments providing a quick knock down. DDVP, therefore, eventually could take the place of the presently recommended TEPP, which is more toxic to mammals.

Diazinon was the most promising material tested. Diazinon is less toxic to mammals but was superior to parathion against flies in these tests. In orchard tests, Diazinon consistently proved to be slightly superior to parathion at equal dosages.

Some growers consider the phosphate insecticides dangerous and prefer not to use them in spite of the additional control of mites, aphids, and San Jose scale that some provide. Methoxychlor wettable powder was the first post-war insecticide recommended for control of the cherry fruit fly in Washington (Frick 1950). As the emulsifiable formulations of methoxychlor were not giving satisfactory results, laboratory and field tests were made by Johansen *et al.* (1954). Their results showed the poor contact action of methoxychlor and were confirmed by the further testing reported herein. The search for a more suitable chlorinated hydrocarbon insecticide was begun. Perthane is considered the most promising. Although it exhibits lower contact action than aldrin or heptachlor, Perthane has an extremely low mammalian toxicity and is therefore desirable on a short-season fresh fruit such as cherries.

Wettable powder formulations cannot be used as satisfactorily in the spray chamber's atomizing apparatus. No attempt to adapt the chamber to the powders has been made because of the limited use of powder formulations in control programs. The cherry industry objects to the visible residues remaining on the fruit from wettable powder formulations.

SUMMARY.—The contact action of 18 insecticides was compared, using a Hoskins-Caldwell spray chamber and the cherry fruit fly as a test insect. Of the phosphate insecticides, DDVP, Diazinon, Pirazinon, Phosdrin, and demeton each had an LD_{95} index higher than parathion. TEPP and Am. Cyanamid 4124 were slightly less toxic

than parathion. EPN, Dipterex, and malathion were inferior to parathion.

Among the non-phosphates, aldrin and heptachlor were close to parathion at the LD_{95} index. Perthane, chlordane, Dilan, Strobane, and two formulations of methoxychlor were inferior. Perthane was the most effective of this group, being between one-fifth to one-fourth as toxic as parathion. Of the tested materials, Diazinon and Perthane were the most promising for orchard control programs. Diazinon has proved to be slightly superior to parathion in orchard tests at a dosage equal to parathion, while Perthane has given good control of the cherry fruit fly at two to four times the dosage of parathion required.

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Preliminary Tests Using *Bacillus thuringiensis* Berliner against Hornworms[1]

ROBERT L. RABB,[2] EDWARD A. STEINHAUS,[3] and FRANK E. GUTHRIE[2]

At present, more insecticide is applied in North Carolina for the control of hornworms than for the control of any other tobacco pest. Both the tobacco hornworm, *Protoparce sexta* (Johan.), and the tomato hornworm, *Protoparce quinquemaculata* (Haw.), are found on tobacco, but the former is by far the more important. Hornworms can be successfully controlled with a number of different insecticides, but the use of chemicals on tobacco presents a number of problems that may assume greater importance in the future. In particular, heavy applications of certain insecticides may result in chemical residues, which may in turn have undesirable effects on the quality of the finished product. It seems worthwhile, therefore, to investigate any new material or method for control which offers the possibility of reduced chemical residues.

Bacillus thuringiensis Berliner, while harmless to humans, livestock, and plants, has shown promise in the control of several lepidopterous pests (Steinhaus 1951, Tanada 1956). A closely related species, *Bacillus sotto* Ishiwata, has been shown by Angus (1956 a & b) also be to pathogenic for a number of lepidopterous larvae including hornworms. The purpose of the present paper is to report on preliminary laboratory and field tests in which *B. thuringiensis* spore suspensions were used against *P. quinquemaculata* and *P. sexta*. The tests with *P. quinquemaculata* were conducted by personnel of the Laboratory of Insect Pathology, Department of Biological Control, University of California, Berkeley, and the tests with *P. sexta* by personnel of the Entomology Faculty, Division of Biological Sciences, North Carolina State College, Raleigh.

MATERIALS AND METHODS.—*Tests with P. quinquemaculata.*—The spores of *B. thuringiensis* used in these tests were produced at the University of California in 1954 and early 1955 on solid nutrient agar, by methods that have been previously described (Steinhaus 1951, Hall 1954). They had been held at room temperature as a dried powder since that time. When used in the hornworm experiments this material contained approximately 40 billion viable spores per gram.

Laboratory tests were designed to test the general susceptibility of the tomato hornworm to *B. thuringiensis.*[4] A total of 40 insects were fed an aqueous suspension of spores, and 10 others were injected with it. Various concentrations were used in five experiments, in each of which there were 10 treated and 10 untreated insects. In one feeding experiment, the dosage varied from 0.03 to 0.15 ml. of an aqueous suspension of spores of 1 gm./400 ml. concentration when the insects were fed directly by pipette. In another feeding experiment, small tomato fruits were dipped in spore suspensions of the same concentration. Triton B-1956 (Rohm & Haas Co.), a wetting-spreading agent, was added at a concentration of 1:800. In three additional experiments the insects were placed on potted tomato plants which had each been sprayed with about 125 ml. of the spore suspension.

In the field trial with *P. quinquemaculata* a small plot of about 10 plants was established in a tomato field near Patterson, California. An infestation of about 12 hornworms were on these plants. This plot was sprayed with a gallon of water in which 1 gram of spores had been suspended.

Tests with P. sexta.—The *B. thuringiensis* spores used in these tests were of two types, arbitrarily designated as "old spores" and "new spores." The "old spores"

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[2] Assistant Professor, N. C. State College, Raleigh.

[3] Professor of Insect Pathology, Department of Biological Control, University of California, Berkeley.

[4] The authors wish to thank Mariece M. Batey for her assistance in the performance of these tests, as well as Earl R. Oatman who assisted in making the field trial.

were from a lot produced at the University of California in 1954, on nutrient agar. The "new spores" were produced at the University in June, 1956, in aerated nutrient-broth cultures. Both preparations contained an abundance of the characteristic crystal formed in the sporulating bacillus, and believed to be responsible for its toxicity (Hannay 1953, 1956, Steinhaus & Jerrel 1954). The "old spore" powder consisted of approximately 40 billion viable spores per gram; while the "new spore" powder contained approximately 50 billion viable spores per gram, as determined by nutrient-agar plate counts.

Three small-plot field tests were conducted during the first week of August, 1956, at the Central Crops Research Station near Clayton, North Carolina. Each spore suspension used in tests 1 and 2 was made by mixing a given quantity of spores in a pint of water with a Waring blendor. This spore concentrate was then diluted to desired strength by the addition of water. The spore suspensions used in test 3 were made by merely adding the spore powder directly to water in the spray tank. Triton B-1956 was added to the spore suspensions at the rate of 1:2000.

There were two treatments, other than the spore suspensions, included in the tests. Treatment 5 of test 1 was a spray prepared from 25% TDE emulsifiable concentrate applied at the rate of about 0.75 pound of TDE per acre. Treatment 1 of test 2 consisted of a spray suspension made by blenderizing two fifth-instar larvae which had died from an application of spore material in test 1. All check plots were sprayed with water containing Triton B-1956 at 1:2000.

Applications of all materials were made with a 3-gallon, compressed air sprayer. The spray nozzle was moved in an arc from the top to the lower leaves of both sides of each plant in order to insure good coverage. Most of the applications were at the rate of 50 to 60 gallons per acre.

The tobacco plants utilized were from 5 to 6 feet tall and had been topped. Four or five leaves were removed from the base of each plant in order to facilitate larval counts. A randomized block design was employed for all three experiments. Treatments were replicated four times in experiment 1 and three times in experiments 2 and 3. Each plot consisted of ten staked plants separated from adjacent plots by two or more guard rows. Although there was an extremely heavy natural infestation of hornworms, some larvae were carefully moved into the plots from adjacent plants, to contribute to uniformity of infestation among plots.

A pre-treatment count of eggs and larvae was made only in experiment 1. Post-treatment counts of normal, moribund, and dead larvae were made at the intervals recorded in tables 1, 2, and 3. Abnormally sluggish larvae with diarrhea were recorded as moribund. The anal area and anal prolegs of such larvae were soiled with excrement. Upon death, disintegration of internal tissues and discoloration proceeded rapidly, but the integument remained intact as shown in figure 1. The instar of each larva observed was recorded.

RESULTS.—*Tests with P. quinquemaculata*—The 1955 infectivity tests using the tomato hornworm, showed this insect to be susceptible to all dosages (as low as 0.01 ml.) when the *B. thuringiensis* spore suspension was introduced into the body cavity of the larva by injection or by puncturing the insect's integument with a spore-laden needle. Similarly, larvae became mortally infected when the spore suspension (doses as low as 0.03 ml.) was fed directly by pipette. In the experiment in which the hornworms were fed tomato fruits that had been dipped in the spore suspension, all 10 insects died of the bacterial infection. In the three experiments in which larvae were placed on potted plants sprayed with spore suspensions, from 90 to 100% of the larvae died of infection.

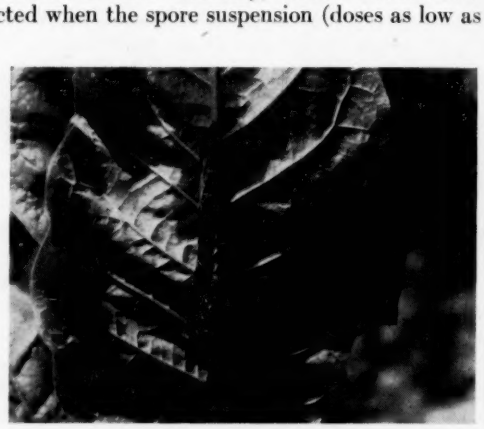

FIG. 1.—A fifth-instar tobacco hornworm, killed by *Bacillus thuringiensis* Berliner.

Table 1.—Results of test 1, showing the effects of *Bacillus thuringiensis* and TDE on tobacco hornworms and budworms.[a]

No.	Treatment, Amount per Gallon	Rate (Gal./A.)	Hornworm (Instars 2–5)								No. Living Budworms at 67-Hrs.
			Pre-treatment Count	Per Cent Reduction of Normal Larvae			No. and Condition of Larvae Observed[b] at 67-Hrs.			No. of Normal Larvae 7-Days	
				20-Hrs.	44-Hrs.	67-Hrs.	Normal	Moribund	Dead		
1	New spores, 1 gm.	49	81	−11.8	28.2	74.6	21	11	39	—	25
2	New spores, 2 gm.	54	79	0.1	60.2	88.6	9	14	47	6	27
3	Old spores, 1 gm.	55	84	2.0	38.2	75.3	21	9	48	—	9
4	Old spores, 2 gm.	55	89	13.1	80.1	96.0	4	10	69	—	17
5	25% TDE, 28.4 ml.	49	83	59.4	86.4	92.8	6	0	15[b]	3	6
6	Water (check)	55	81	−21.2	−20.8	−28.3	101	0	0	128	23
	L.S.D. at 5% level			16.4	25.9	10.8[c]					

[a] Four replications; 10 plants per plot.
During the 7-day testing period, the average maximum and minimum temperatures were 87° and 65° F., respectively. Rainfall was 0.06 inch on the fourth day and 0.25 inch on the seventh day.
[b] Larvae observed on plants. Those affected by TDE fell from plants more readily than those affected by the bacterium.
[c] Use this figure for comparisons among the first five treatments only. Due to the high variability within check counts, it was necessary to eliminate them from the analysis.

Table 2.—Results of test 2, showing the effects of a suspension of blenderized diseased larvae and *Bacillus thuringiensis* spores on tobacco hornworms.[a]

No.	Treatment: Description	Rate (Gal./A)	No. of Normal Hornworms (Instars 2–5) After: 48-Hrs.	72-Hrs.
1	Diseased 5th-instars,[b] 2 per gal.	58	122	109
2	Old spores, 2 gm. per gal.	60	10	7
3	Water (check)	71	57	51

[a] Three replications; 10 plants per plot.
The average maximum and minimum temperatures during the testing period were 89° and 63° F., respectively. Rainfall was 0.25 inch on last day.
[b] From plots treated with *Bacillus thuringiensis* spores in test 1.

Table 3.—Results of test 3, showing the effects of new and old *Bacillus thuringiensis* spores at 4 and 2 grams per gallon, respectively, on tobacco hornworms.[a]

No.	Treatment: Amount per Gallon	Rate (Gal./A.)	No. of Normal Hornworms (Instars 2–5) After: 24-Hrs.	48-Hrs.	72-Hrs.
1	New spores, 4 gm.	60	43	7	4
2	Old spores, 2 gm.	71	65	18	11
3	Water (check)	58	91	88	105

[a] Three replications, 10 plants per plot.
The average maximum and minimum temperatures during the testing period were 87° and 67° F., respectively. No rainfall.

In the field trial with *P. quinquemaculata*, 12 fourth- or fifth-instar larvae were on the plants used. Of these, 2 pupated before the completion of the experiment, but all of the remaining 10 died of infection by *B. thuringiensis*. In all instances the cause of death was confirmed by microscopic and cultural examinations.

Tests with P. sexta.—Results of test 1 are presented in table 1. After 20 hours, the TDE spray (treatment 5) had reduced the number of normal appearing larvae far more than any of the *B. thuringiensis* treatments. By the time 44-hour counts were made, all of the treatments had reduced larval populations. At this time, there was no significant difference in the effect of the old spores at 2 grams per gallon and the standard TDE treatment. Also, the higher rates of both sources of spores were significantly more effective than the lower rates. On the basis of the 72-hour count, the higher rates of both new and old spores had performed as well as TDE and significantly better than the lower concentrations of spores. A 7-day check showed that larval populations were still at a very low level in plots receiving applications of new spores at the rate of 2 grams per gallon and those treated with TDE, whereas a slight increase in population had occurred in the check plots. Although there were no significant differences between the performance of the new and old spores at any of the counts, the data indicate that the old spores were slightly more effective than the new. This was particularly true at the 20-hour count where the difference between the effect of the old and new spores was almost significant at the 5% level. This difference may signify a slight difference in the relative potency of the toxins associated with the two batches of spores, or it may simply be a reflection of the normal variation of mortality sometimes seen in experiments such as these.

In test 1, some data were also collected on budworms (*Heliothis* spp.). Although the data are too few and variable for definite conclusions, they do indicate that the spore applications had no appreciable effect on these insects.

In test 2, as shown in table 2, the suspension prepared from diseased larvae had little if any effect on the larval populations, whereas the old spore suspension was again effective. There was a much heavier larval population in one replication of treatment 1 than in any of the other plots. The failure of the suspensions prepared from freshly dead larvae to cause infection can undoubtedly be explained by the fact that the bacilli, as they existed in the dead larvae, had not had time to form spores (and crystals). It has been shown previously (Steinhaus 1951, Steinhaus & Jerrel 1954) that the vegetative form of the bacillus is noninfectious for most insects, and that it usually takes several days before spores and crystals are formed and the bacillus becomes pathogenic.

In test 3 (table 3), the new spores at 4 grams per gallon and the old spores at 2 grams per gallon gave excellent results. Although the data indicate that the new spores at twice the concentration of the old spores gave a slightly greater reduction in hornworms, this conclusion is not supported by statistical analysis. One factor which may have contributed to this lack of difference between the high and low concentrations was that the lower concentration was applied at about a 15% greater rate than the higher concentration.

Supplementary to test 3, an attempt was made to ascertain the amount of feeding by larvae after treatment with a spore suspension. Each of 10 undamaged plants was infested with two large fourth- or early fifth-instar larvae. Five of these plants were immediately sprayed with the new spore suspension comprising treatment 1 of test 3. The other five plants were sprayed with water. After 7 days, less than 1 leaf had been eaten on the 5 treated plants as compared with a loss of 21 leaves on the 5 check plants. These data indicate that hornworms immediately stop feeding after treatment with *B. thuringiensis*.

To be certain that the mortality of the hornworms was the result of infection by *B. thuringiensis*, representative larval cadavers were sent to the University of California for diagnosis. In all instances it was confirmed, by microscopic and cultural tests, that the larvae had been killed by infection with *B. thuringiensis*.

SUMMARY.—Preliminary tests were conducted to investigate the possibility of using *Bacillus thuringiensis* Berliner as a control agent for the tomato hornworm, *Protoparce quinquemaculata* (Haw.), and the tobacco hornworm, *P. sexta* (Johan.).

Infectivity tests conducted in 1955 at the University of California showed *P. quinquemaculata* to be markedly susceptibile to infection by *B. thuringiensis* when the bacillus was administered perorally or by injection. A limited field trial indicated that the bacillus is capable of effectively killing this hornworm on tomatoes.

Small plot field tests utilizing the bacillus against *P. sexta* on tobacco were conducted in North Carolina. On the basis of counts made 72 hours after treatment, the spore suspension at the concentration of 2 grams per gallon gave as good a result as TDE, a recommended hornworm toxicant. Although the insecticide gave a quicker reduction in larvae, this reduction may be of

minor importance, since larvae treated with the spore material ceased feeding almost immediately after treatment. Limited observations indicated little effect on budworms (*Heliothis* spp., primarily *virescens* (F.)) by the application of spores of *B. thuringiensis*.

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Plot Tests of Potential Acaricides at Camp Bullis, Texas, in 1954[1]

GEORGE W. HUNTER, III,[2,3] F. M. PHILIPS, JAMES V. GRIFFO, JR. and J. S. WILLIAMS[4]

Fourth Army Area Medical Laboratory, Brooke Army Medical Center, Ft. Sam Houston, Texas

The paucity of knowledge of the biology of the lone star tick, *Amblyomma americanum* (L), has precluded the development of an effective acaricide even though the tick is widely distributed throughout the southern and eastern United States, extending as far north as Newfoundland. In 1952 it was decided to undertake a series of field plot tests at Camp Bullis, Texas on various potential acaricides in the hope of finding effective compounds for the control of this pest in the field. This program was coordinated with that of the U. S. Department of Agriculture which was doing some similar testing of chemicals on *A. americanum* in the southeastern United States.

The results of the 1952 tests indicated that dieldrin was the best material of those tested (Therrien *et al.* 1953, 1954). Dieldrin was retested in 1953 and its efficacy compared with other compounds. Once again dieldrin was found to be the best (Hunter, *et al.* 1954a, 1954b). In the following year dieldrin underwent further testing both as a dust and as a spray together with other compounds heretofore untested in the Camp Bullis area. The results of these experiments are reported in this paper.

MATERIALS AND METHODS.—In order to facilitate comparisons of the results obtained in 1952 and 1953 the same sized plots, techniques of mixing, applying the chemical and making the counts were utilized (Therrien *et al.* 1954, Hunter *et al.* 1954b). Two 9-square-foot quadrats were selected and marked in each 0.025-acre plot. These were used for counts each time, both before and after treatment.

The dusts were applied at dawn using pyrophyllite as a diluent for them. Varying amounts of the toxicant were applied by altering the percentage of diluent in the dust and by using a standard type rotary duster.

For the first time both dieldrin and endrin were also tested by us as a spray. In such cases xylene was used as a solvent, Triton X-155 as an emulsifier, and water as a diluent. Six undusted plots, plus 3 others (3 that were treated with the diluent only and 3 that were sprayed minus the toxicant) were selected as controls for each phase and counted in the same manner as the test plots. Counts were made on two quadrats in each control and treated plot on the day prior to the application of the dust or spray and thereafter at the end of 1, 2, 7, 14, 21 and 28 days. The degree of control was calculated by Abbott's formula (1925). As in the past all plots were treated in the early morning hours to overcome interference from the wind.

RESULTS AND DISCUSSION.—Toxaphene, endrin and dieldrin were the only chemicals tested during the summer of 1954. The results of these 1954 experiments at Camp Bullis, Texas on potential acaricides against the lone star tick are summarized in table 1. Endrin spray at the rate of 1 pound per acre afforded the best knockdown at the end of 24 and 48 hours and gave slightly better over-all results than the dieldrin spray used at the rate of 0.5 pound per acre. Both of the sprays were somewhat more efficacious than dieldrin used as a dust. Toxaphene applied as a dust at the rate of 2 pounds per acre gave almost as good results as dieldrin dust at 0.5 pound per acre. The spray controls alone gave between 22.9 and 56.4% reduction (table 1).

From the standpoint of both initial kill and residual effect dieldrin gave the best results in 1952. When used as a dust at 0.5 or 1.0 pound per acre dieldrin gave 96.3 and 98.7% reduction in 48 hours and 97.7 and 99.6% reduction for as long as 4 weeks. These results were superior to those achieved with aldrin and lindane or the DDT and chlordane controls. The best results secured with the controls was with DDT used at 4 pounds per acre and which gave 90.3 and 97.5% reduction in 48 hours and 4 weeks, respectively (Therrien *et al.* 1953).

In 1953 Sulphenone, Neotran, heptachlor and N-butylacetanilide were compared with dieldrin and DDT. Again under the environmental conditions encountered at Camp Bullis, Texas, none of the compounds except dieldrin gave satisfactory results. Dieldrin at 0.5 and 1.0 pound per acre gave 96 and 98% reduction in 48 hours

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[2] Affiliated Units of the Graduate School, Baylor University, Waco, Texas.

[3] Current address: Dept. of Microbiology, College of Medicine, University of Florida, Gainesville, Florida.

[4] The authors express their appreciation to the following who contributed to the development of this project: D. E. Kotler, J. M. Padilla and D. E. Shamer.

Table 1.—Control obtained with various formulations of dusts and sprays applied against the lone star tick at Camp Bullis, Texas, during 1954.

FORMULATION	NUMBER OF PLOTS AND QUADRATS[a]	PRETREATMENT COUNTS	PERCENTAGE REDUCTION[b]					
			24 Hrs.	48 Hrs.	1 Wk.	2 Wks.	3 Wks.	4 Wks.
Toxaphene dust								
1.0 lb/acre[c]	3–6	301	44.5	69.6	69.8	89.6	88.7	94.0
2.0 lb/acre	3–6	229	81.1	83.3	93.4	92.2	94.2	94.1
Endrin spray								
0.5 lb/acre	3–6	859	94.7	96.7	95.5	98.9	99.4	100.0
1.0 lb/acre	2–4	318	99.3	95.3	99.7	100.0	100.0	100.0
Dieldrin spray								
0.25 lb/acre	3–6	306	90.9	92.9	95.0	97.8	100.0	97.6
0.5 lb/acre	3–6	470	89.8	93.0	99.1	98.5	98.3	100.0
Dieldrin dust								
0.25 lb/acre	3–6	515	73.3	95.5	94.8	93.8	95.7	89.3
0.5 lb/acre	3–6	595	82.6	89.4	93.3	97.3	94.5	94.4
Check (Sprays)								
Phase VIII	3–6	277	30.0	25.5	56.4	25.3	22.9	—
Phase IX	3–6	309	—	—	—	—	—	3.8
Check (Dusts)								
Phase VIII	3–6	362	—	10.4	—	—	—	20.4
Phase IX	3–6	198	—	3.8	—	—	—	—
			Percentage of Pretreatment Total Present					
Check (Untreated)								
Phase VIII	6–12	888	71.2	64.4	51.6	73.0	52.9	38.2
Phase IX	6–12	900	83.1	72.9	92.3	100.8	67.1	52.2

[a] The first figure represents the number of plots and the second figure represents the number of quadrats.
[b] The per cent reduction was determined through the use of Abbott's formula (1925).
[c] Phase VIII is the check for the first formulation for each compound test, and Phase IX is the check for the second formulation.

and 99 and 97% reduction, respectively, at the end of 4 weeks (Hunter *et al.* 1954).

During the 1954 tests dieldrin did not yield as good results as in the previous years. Used as a dust at 0.5 pound per acre it provided only 89.4 and 94.4% reduction at the end of 48 hours and 4 weeks, respectively (table 1). Dieldrin spray applied at the same concentration gave better results as there was a reduction of 93% in 48 hours and the control achieved during the first 4 weeks ranged from 98.5 to 100% (table 1). This loss of efficiency in dieldrin dust is difficult to understand as the temperatures, rainfall and humidity did not differ markedly during the three summers when this chemical was under test.

The results of the 1954 plot tests indicate that endrin spray applied at the rate of 0.5 or 1.0 pound per acre was slightly superior to dieldrin.

There may be several practical applications of this work: (1) Dieldrin or endrin may be used to protect small groups of men forced to work or live in an area heavily infested by the lone star tick; (2) Since the cost is not excessive, it is possible that these compounds might be used to treat large grazing areas by dusting from small planes or helicopters in cases where difficulty is experienced in controlling ticks on cattle; (3) It is also possible that if properly supervised and applied dieldrin or endrin *might* be used safely on lawns that have become badly infested, either through propinquity to known infested areas or by ticks from pets, especially dogs. In this connection it would be necessary to first test the efficacy of these chemicals against the American dog tick, *Dermacentor variabilis* (Say), although in the area of San Antonio, Texas, the lone star tick is common on dogs.

CONCLUSION.—On the basis of the work conducted during 1952–54 it appears that both dieldrin dust at 0.5 or 1.0 pound per acre, or the spray at 0.5 pound per acre and endrin spray at 1.0 pound per acre, will provide 96% or more reduction in the population of the lone star tick. *Amblyoma americanum* (L.), within a period of 48 hours following application and that this reduction will be maintained for at least 4 weeks. Obviously one cannot generalize, as different environmental conditions in other parts of the world, such as rainfall, humidity, etc., may prevent duplication of the results achieved at Camp Bullis, Texas. However it is believed that in a hot dry environment, with low humidity, such as is found at Camp Bullis, at least a marked temporary reduction in the lone star tick population will result if either dieldrin or endrin is used at 0.5 and 1.0 pound per acre, respectively.

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The Dispersion of Radioactively Tagged White-Pine Weevils in Small Plantations[1]

P. A. Godwin,[2] H. A. Jaynes,[2] and J. M. Davis,[3] *Forest Service, U.S.D.A.*[4]

The ability of an insect to disperse freely and widely is an important factor in the design of control measures to be used against it. This is particularly true of a relatively long-lived insect like the white-pine weevil, *Pissodes strobi* (Peck). In the Northeast the weevil begins to emerge from hibernation by mid-April, and oviposition commences almost immediately and continues until mid-June.

Spraying by aircraft has usually been timed to coincide with the first appearance of the weevil in the spring (Connola *et al.* 1955). If the insecticide applied has a relatively short residual effect, then sufficient time remains for an assiduous flier to reinfest plots in which the resident population has been controlled. In spraying with ground equipment the greater deposit of insecticide provides longer residual protection, allowing reliable estimates of the suppression of the initial population; but the problem of reinfestation in the long run is still present.

Reports in the literature on the flight capabilities of the white-pine weevil are contradictory. Pierson (1922) states, "It is also apparent that flight is seldom resorted to"; but later writes, "Evidence all points to the fact that the beetles usually fly over the plantations before egg laying." Graham (1926) felt that, though the insect is a strong flier, it rarely flew and that there was a short flight period in early spring during which the weevils became widely disseminated. One hundred and twenty-five flights were observed by Barnes (1928). He found the weevil to be a strong flier with flights of over 300 feet. Plummer & Pillsbury (1929), using Barnes's methods, were unable to reproduce his results. Their observations were similar to Graham's. MacAloney (1930) believed that under optimum conditions of 75° to 80° F. flights of several miles were possible.

The present study attempted to clear some of this confusion and help provide a basis for establishing the degree of isolation required for small experimental spray plots.

Materials and Methods.—Weevils for the study were obtained in July 1954 by collecting infested terminals of white pine and allowing the adults to emerge in the insectary. The weevils were than held in battery jars until treated on September 2.

The plantation in which the release was made was roughly rectangular, extending 900 feet east and west and 600 feet north and south. The area was bounded on the north and east by mature second-growth hardwoods; on the south by alder brush; and to the east by a 50-foot band of hardwoods 50 to 60 feet tall, beyond which were other plantations of white, red, and jack pine. The pines in the plantation were 5 to 7 feet tall, planted 6×6 feet. Numerous clumps of wild cherry and alder occurred in the plantation. Since much of this hardwood brush was concentrated in the western end, the actual release point was about 200 feet east of the plantation's center.

The isotope material used was $Sc^{46}Cl_3$ in a 0.52N, HCl solution with a concentration of 46.45 mc/ml. For tagging the weevils 3.8 ml. of 20% ethynol were added to 0.7 ml. of the stock isotope solution.

The weevils were tagged in the field close to the point of release. The manipulations involved in treating the weevils were carried on behind a steel brick shield using apparatus designed by Davis and Nagel and described elsewhere (Davis & Nagel 1956). The weevils were treated in 13 groups of 100 each until diminishing isotope supply required smaller groups. A total of 1,600 weevils was treated.

After treatment each group was put in a petri dish and placed under a pine so that the weevils might escape as they recovered. Twenty-four hours after treatment only 10 dead weevils were found in the dishes. Precision Instruments model 111 Scintillators were used to detect individual weevils after release. Beyond the immediate vicinity of the release trees, where background counts were normal, individual weevils could be detected at 4 to 5 feet.

Fig. 1.—Distribution of weevils October 13, 1954. +—trees on which weevils were released. •—trees to which tagged weevils moved.

Results.—Few weevils moved from the release trees in the autumn of 1954. The area was examined September 7–10, September 20–23, and October 11–13. By October 13 only 12 weevils had moved from the release trees, although 60 to 70% of the treated weevils were still active on these trees (fig. 1).

The plantation was again examined in 1955 during the period April 13–15. Only three weevils were found on tree leaders, but active spots were found under trees which had had weevils on them in October 1954. Winter mortality proved to be very high. On April 22 the whereabouts of 109 living weevils was known—the largest number recovered during 1955.

On April 18, 20, 21, and 22 maximum air temperature reached or exceeded 70° F. The 6 days following, during

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[2] Northeastern Forest Experiment Station, New Haven, Conn.

[3] Agricultural Research Center, Beltsville, Md.

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which the area was again checked, were characterized by cool, wet weather with a maximum of 66° on the 28th. Since 70° F. was the temperature reported necessary for flight (Barnes 1939, MacAloney 1930), it is felt that the dispersion of the weevil, as plotted for April 28, was the result of movement taking place on April 18, 20, 21, and 22 (fig. 2).

The final examination of the area was made May 11–13. Maximum temperatures in the intervening period ranged in the 80's. During the examination period temperatures were again cool—a maximum of 68° F.—preventing much change in the dispersion pattern while the examination was in progress (fig. 3).

DISCUSSION.—This study supports the observations of Barnes that the weevil flies well and frequently, when ever temperatures are favorable. Of the 109 weevils

FIG. 2.—Distribution of weevils April 28, 1955. +—trees on which weevils were released. •—trees to which tagged weevils moved.

FIG. 3.—Distribution of weevils May 13, 1955. +—trees on which weevils were released. •—trees to which tagged weevils moved.

located April 22, 31 had already left the release trees and by April 28 an additional 29 weevils had moved into the plantation. Of the 60 weevils plotted April 28, 49 had again moved by May 11 and 17 more had entered the field from the release trees. While the length of individual flights were not measurable with the detection method used, the distinct stratification of the April 28 distribution pattern suggests that weevil movement to the plantation edge—distances of 300 to 400 feet—was the result of direct uninterrupted flight from the release trees.

The movement of the weevils studied here indicates that experimental plots should be separated from infested areas by a minimum of 800 feet. The small number of weevils involved makes estimates of the frequency of various flight lengths hazardous, but movement of 724 feet is a certainty and at a frequency of 1:100 flights of this length would be common in natural populations. In south-central New York State, in the vicinity of the study area, infestations of 2,000 to 3,000 weevils per acre are discouragingly frequent (Northeastern Forest Experiment Station 1954).

The study shows that failure to remove a few infested leaders which might occur after a spray operation will result in the plantation being generally infested after emergence and that the practice of spraying a few trees surrounding the infested trees to remove this residual population is of doubtful value.

The assemblage of weevils along the hardwood edges would seem to support the growing evidence that in addition to shade, a hardwood overstory acts as a mechanical barrier which the weevil is reluctant to penetrate.

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Tests of Bark-Penetrating Insecticides to Control the Douglas-Fir Beetle[1]

ARCHIE L. GIBSON, *Intermountain Forest and Range Experiment Station, Forest Service, U.S.D.A., Ogden, Utah*

The Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopk.) is probably the most destructive insect enemy of Douglas-fir forests in the western United States. In the past 25 years, outbreaks of this insect have caused serious depletion of this important timber species in many stands. Forest entomologists, foresters, and lumbermen have noted and reported these losses, but until recent years, because of the low stumpage value of Douglas-fir, the insect damage caused little public concern. Rising stumpage values and a realization of the importance of this timber in both national and local economies have created a need for methods to reduce losses caused by this insect.

The late W. D. Bedard's study of the Douglas-fir beetle (Bedard 1950) established the major facts concerning the life history of this insect, gave a résumé of the control measures tested up to 1945, and other important information. Of the control methods tested previous to 1945, felling and burning of infested trees was the only one which had proved effective. Under the most favorable conditions, control by means of fire was highly effective. Under certain conditions, however, there was the danger of causing a forest fire or of scorching nearby trees sufficiently to make them more susceptible to beetle attack. Furthermore, the various phases of the operation —felling the infested trees, cutting them into logs, and decking and burning the logs—became prohibitively expensive with increasing labor costs. Consequently a need arose for cheaper and more practicable means of control.

In 1949 exploratory tests to control the Douglas-fir beetle with bark-penetrating insecticides were initiated at the Coeur d'Alene Forest Insect Laboratory (Gibson 1952). These had been found highly effective in killing broods of the mountain pine beetle, *D. monticolae* Hopk., (Gibson 1943) in both lodgepole and western white pines. An epidemic infestation of the Douglas-fir beetle on the Kootenai National Forest in Montana provided opportunity for the exploratory tests. The area appeared typical of Douglas-fir stands in the region and its accessibility added to the desirability of the site. Later, additional tests were established in infested areas on the Coeur d'Alene and St. Joe National Forests in Idaho.

MATERIALS AND METHODS.—The immediate objective of the tests was to determine the bark beetle brood mortality resulting from application of certain bark-penetrating insecticides. Insecticidal formulations selected for the tests were five Diesel oil solutions and three emulsions of several chemicals known to have fumigant properties (table 1). The oil solutions were

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selected on the basis of good previous results; the emulsions because they could be conveniently mixed in the field using available stream water.

Insecticide tests were made on two types of infested host material; standing trees and logs. It was hoped that the treatment of infested trees without felling them might prove effective and thus eliminate the costs of felling and bucking them into logs.

In all tests the insecticides were applied with a $3\frac{1}{2}$-gallon hand-operated, pressure-type garden sprayer. The spray, of cone-shaped pattern, was applied until the surface of the bark glistened and the insecticide began to run. In the case of logs, the upper surface was first treated, then the log was turned until an unsprayed portion was uppermost. This side was then sprayed and the turning and spraying repeated until the entire circumference had been treated.

Another study objective was to determine the effect of rain-soaked or snow-covered bark on the penetrability of the insecticides. Water was sprayed on some infested logs until it ran freely from the bark surface and treatment with the insecticides followed immediately. On other logs, spray was not applied until rain for a number of days had thoroughly soaked the surface and was continuing at the time of treatment. The most severe test of spray effectiveness occurred with logs subjected to heavy rain for a couple of days followed immediately by a 3-inch snowfall. The snow was removed with a heavy-bristled shop broom and the logs were then treated. Data from material sprayed under such conditions were expected to indicate the amount of insect mortality that could be expected after inclement weather normally encountered during a control project.

After spraying, a lapse of time was allowed for the spray to affect the infesting broods. Samples of bark were then removed so that counts of living and dead beetle brood by developmental stages per unit area of bark could be made.

RESULTS.—The application of oil solutions S-1, S-2, and S-3 caused 100% mortality of Douglas-fir beetle broods in dry standing infested trees or logs (table 2). When the treated material was wet, or the cambium green, acceptable but less then 100% control was achieved. Formula E-1 permitted less than 6% survival of infesting broods on both dry and wet tests.

Table 1.—Insecticide formulations used.

Formula Number	Insecticide	Synthetic Detergent	Diesel Oil
Oil Solutions			
S-1	Orthodichlorobenzene, 1 part	—	3 parts
S-2	Orthodichlorobenzene, 1 part	—	5 parts
S-3	Trichlorobenzene, 1 part	—	5 parts
S-4	Dichloroethyl ether, 1 part	—	5 parts
S-5	Ethylene dibromide, 1.5 lbs.	Triton X-100 8 oz.	4.75 gals.

Formula Number	Insecticide (lbs.)	Synthetic Detergent (oz.)	Diesel Oil (lbs.)	Water (gal.)
Emulsions				
E-1	Ethylene dibromide, 2	Triton X-100, 3 Triton B-1956, 5	5	4.125
E-2	Ethylene dibromide, 2	Triton X-100, 3 Triton B-1956, 5	4	4.25
E-3	Ethylene dibromide, 2	Triton X-100, 3 Triton B-1956, 5	6	4

Table 2.—Toxicity of bark-penetrating insecticides against Douglas-fir beetle broods.

Formula Number	Type	Bark Condition	Treated Logs: Number Examined	Treated Logs: Per Cent Mortality	Treated Standing Trees: Number Examined	Treated Standing Trees: Per Cent Mortality
S-1	Oil solution	Dry	31	100	11[a]	100
		Wet	9	82.4		
S-2		Dry	25	100	232[a]	100
		Wet			15[b]	100
S-3		Dry	3	100	9[a]	100
S-4		Dry			5[a]	100
S-5		Dry	14	100		
		Wet	15	86.5	14[b]	100
E-1	Emulsion	Dry	16	98.0	8	99.2
E-2		Wet	16	94.2		
		Dry	7	100	4[a]	82.4
E-3		Dry	7	100		
	Untreated material		43	37	44	29

[a] Treated from ground to 5 feet above.
[b] Treated from ground to 20 feet above.

Based on effectiveness and cost, ethylene dibromide emulsion is the best formulation to use where control is contemplated in remote areas involving high transport costs. Not only can excellent control be expected both in dry standing trees and logs, but it is apparently superior to oil-base formulas on wet material.

The bark of the Douglas-fir was found to be highly absorptive of the oil-base insecticides and required from two to three times the quantity of this type of insecticide for surface saturation of a given area as that for either lodgepole pine or western white pine. A gallon of oil-base insecticide adequately saturated an average of about 31 square feet of infested Douglas-fir bark. Emulsions of the same insecticide gave less coverage per gallon.

Green cambium also was found to offer decided resistance to penetration by either emulsions or oil-base sprays. This was indicated by some beetle brood survival in such areas, in contrast to complete mortality where the cambium was dead.

Data in table 2 for brood mortality in untreated material are for the period coinciding with that between the time of treating and the subsequent examination of the sprayed logs and trees.

When treatment was applied in the fall, the insecticide caused some mortality before winter, but a complete measure of spray effectiveness was not obtainable until after a period of warm weather the following spring or early summer. Cause of this delayed lethal effect was not determined but is believed to be due to higher temperatures volatilizing the lethal agents.

On dry host material, excellent control was obtained with all formulas. Even weaker concentrations than those tested might prove effective. Although spraying is a comparatively small fraction of the total cost of control projects, a reduction in the quantity of the more expensive ingredients in the concentrate would lower both material and transport costs.

SUMMARY.—Tests with bark-penetrating sprays were

conducted over a 3-year period in a search for cheaper and more effective methods to control the Douglas-fir beetle. Sprays tested comprised a lethal agent, emulsifying and/or spreading agents, and a water or Diesel oil base. Orthodichlorobenzene, trichlorobenzene, dichloroethyl ether in oil and ethylene dibromide in either Diesel oil or in emulsion form gave equally good control. Water-saturated or green bark prevented complete control with some formulas under the severe conditions set up in the tests. The ethylene dibromide emulsion is preferred due to the ready availability of water on most control projects and the consequent elimination of about 75% of transport costs required for oil-base sprays.

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Insecticides for Control of Peach Insects[1]

MARVIN L. BOBB, *Virginia Agricultural Experiment Station, Piedmont Fruit Research Laboratory, Charlottesville*

Much progress has been made on the control of peach insects during the past 10 years. During this period a large number of organic insecticides have been developed and some of these have given excellent control of peach insect pest (Bobb 1952, 1953). Many of the organic insecticides have a wide range of effectiveness but a few are specific to certain species or groups of insects. Woodside (1950) found that most of the catfacing, which consists of scars bare of pubescence, in Virginia has been caused by the tarnished plant bug, but stink bugs of the genus *Euschistus* and the plum curculio also cause some injury. He advocated the application of a petal-fall spray containing DDT or parathion for control. Chandler (1955) stated that dieldrin was more persistent than DDT for tarnished plant bug and stink bug control.

This paper presents results of experiments on various insecticides for control of the plum curculio, *Conotrachelus nenuphar* (Hbst.), the oriental fruit moth, *Grapholitha molesta* (Busck), and insects causing catfacing on peaches. Although a number of insects may be responsible for causing catfacing of peaches, the tarnished plant bug, *Lygus lineolaris* (P. de B.) was primarily the one involved in these studies.

FIELD CONTROL.—The orchard in which the field insecticidal tests were made contained several varieties of peaches, but all data given in this report are from trees of the Elberta variety. Each of the test plots contained approximately 40 trees, usually 4 rows of 10 trees each.

All sprays were applied with a blower-type sprayer using a 2X concentration. Each tree received from 2 to 2.5 gallons of the concentrated spray at each application. In 1954 three sprays for first brood and one spray for second brood plum curculio were applied. No spray was applied for control of the second generation of the oriental fruit moth. The dates of applying the sprays in 1954 were as follows: shuck-split, April 14; shuck-fall, April 23; first cover, April 30; and third cover, July 2. During 1956 the sprays were applied for plum curculio and oriental fruit moth control on the following dates: shuck-split, April 27; shuck-fall, May 5; first cover, May 12; second cover, June 6; and third cover, July 5. For control of catfacing insects in 1956 only one spray was applied. This was a petal-fall spray applied on April 12.

All data on control are based on examination of the peach fruits at harvest. From 600 to 1000 fruits were picked at random from the trees in the center rows of each plot and carefully examined for insect injuries.

LABORATORY TESTS.—During 1954, 1955, and 1956 eight insecticides were tested on first generation plum curculio adults in the insectary. The beetles were dipped in various insecticide formulations and immediately confined in cages for later observation on mortality. These cages had a wooden bottom, screen-wire sides, and a top which was closed with a piece of cheesecloth held in place by a rubber band. The bottom of each cage was covered with 1 inch of moist sand, and unsprayed peach foliage was added as food for the beetles. Replicated tests were made using 10 beetles per replicate.

RESULTS AND DISCUSSION.—The insecticides used and the percentage of wormy or injured fruits by the plum curculio and the oriental fruit moth for 1954 are given in table 1. Control of the plum curculio with dieldrin was outstanding; while parathion and EPN gave satisfactory control of this insect. All other insecticides tested gave unsatisfactory control of the plum curculio at the dosages used.

No second brood sprays were applied for oriental fruit moth control in 1954, resulting in unsatisfactory control from all insecticides except parathion and EPN which gave a fair degree of control with first brood sprays. One DDT spray during the first brood period did not give control of later broods. During 1955 all fruit was killed by freezing weather, but observations of non-bearing trees which were sprayed indicated that one application of DDT for control of the first brood, followed by a second application at the peak of moth flight of the second brood, markedly reduced the number of infested terminal twigs.

During 1956 all insecticides tested, with the exception of DDT, gave satisfactory commercial control of the plum curculio (table 2). The percentage of curculio infested fruits in the DDT plot was higher than in the check plot. In the check plot, however, 17.3% of the first brood larvae were parasitized by *Triaspis curculionis* (Fitch), whereas in the DDT plot only 1.6% of the larvae were parasitized. Dieldrin was used at half the strength usually recommended but gave excellent control of the plum curculio. For the past 3 years dieldrin has been used

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in small-scale, commercial tests at 0.25 pound of wettable powder (50% active ingredient) per 100 gallons of spray, and has consistantly given excellent control of the plum curculio. There was no difference in the degree of control obtained when 2 or 3 sprays of Guthion were applied for first brood control of the plum curculio and the oriental fruit moth. Both parathion wettable powder and parathion water-base emulsion sprays gave 100% control of the plum curculio in 1956.

By comparing the check plots for plum curculio given in tables 1 and 2, it will be noted that the degree of infestation for the 2 years differed greatly. During 1954

Table 1.—Effectiveness of various insecticides for control of the plum curculio and the oriental fruit moth, 1954.

Insecticide (2X Concentrate)	Pounds or Pints per 100 Gallons	Plum Curculio		Oriental Fruit Moth	
		Per Cent Wormy	Per Cent Reduction from Check	Per Cent Injured	Per Cent Reduction from Check
Parathion, 15%, WP	3	5.9	81.5	1.3	79.3
	4	1.8	94.2	0.0	100.0
Malathion, 25%, WP	4	24.8	21.6	4.5	29.3
EPN, 25%, WP	2	5.0	84.2	1.5	76.6
Methoxychlor, 50%, WP	6	10.7	66.2	2.7	57.8
Dieldrin, 50%, WP[a]	1	0.1	99.7	3.2	49.8
Endrin, 18.5%, EC[a]	2	14.7	53.5	3.6	43.9
Check, no insecticide		31.6	—	6.4	—

[a] 50% DDT wettable powder at the rate of 4 pounds added in the shuck-fall spray.

there was a large, early second brood; whereas, during 1956 there was a moderate, late second generation. The rate of egg development and subsequent deposition partly accounted for this difference. Cage tests have shown that newly emerged first brood adults may develop eggs rapidly in the oviducts and begin depositing them as early as 9 days after emergence, or the eggs may develop slowly and require several weeks or more before being deposited.

Satisfactory control of the oriental fruit moth was obtained with all of the insecticides tested, although a low population of this insect existed in the orchard during 1956. Of the materials tested, however, dieldrin and methoxychlor were the least effective.

Results of field studies on the tarnished plant bug and other insects causing catfacing injury to peach fruits in 1956 are also included in table 2. This phase of the work, however, was carried on in a different orchard block, and only a petal-fall spray was applied. Jarring of peach trees showed the greatest numbers of tarnished plant bugs on the trees from April 10 to 13, the trees averaging 6.8 adults per tree during this period, and the spray was applied on April 12. All insecticides tested gave good to excellent control of the tarnished plant bug and, with the exception of methoxychlor, all insecticides reduced injury by 90% or more.

In laboratory tests on plum curculio adults during the years 1954 to 1956, inclusive, 100% kill of first generation

Table 2.—Effectiveness of various insecticides for control of the plum curculio, the oriental fruit moth, and insects causing catfacing of peaches, 1956.

Insecticide (2X Concentrate	Pounds or Pints per 100 Gallons	Plum Curculio (Per Cent Wormy)	Per Cent Injured by	
			Oriental Fruit Moth	Catfacing Insects
Parathion, 15%, WP	3.0	0.0	0.0	1.1
Parathion, 42%,[a] water emulsion	1.0	0.0	0.0	—
Chlorthion, 25%, WP	3.0	1.2	0.0	—
Guthion, 25%, WP[b]	1.5	0.0	0.2	—
Guthion, 25%, WP	1.5	0.0	0.0	—
Malathion, 25%, WP	6.0	1.2	0.2	0.7
EPN, 25%, WP	2.0	0.0	0.0	1.2
Dieldrin, 50%, WP	0.5	0.2	1.9	0.4
Methoxychlor, 50%, WP	6.0	0.0	1.3	2.5
DDT, 50%, WP	4.0	20.0	0.2	0.7
Untreated		14.5	2.4	12.6

[a] Manufactured by Stauffer Chemical Company; stated to contain no oil solvent.

[b] Shuck-fall spray omitted.

adults was obtained within 5 days after treatment with the following materials and amounts per 100 gallons: 15% parathion, WP, 1.5 pounds; 42% parathion, water-base emulsion, 0.5 pint; 50% dieldrin, WP, 0.25 pound and 0.5 pound; 25% malathion, WP, 3 pounds; and 25% Diazinon, WP, 1 pound and 2 pounds. Thimet (48.5%), EC, 2 pints and 4 pints gave 80% and 90% kill, respectively. Demeton, 1 pint and 2 pints, gave no appreciable kill.

SUMMARY.—Field and laboratory studies of insecticides for control of the plum curculio, the oriental fruit moth, and insects causing catfacing on peaches are presented for the years 1954 to 1956, inclusive. Dieldrin gave outstanding control of the plum curculio and of catfacing insects, but was ineffective against the oriental fruit moth. Parathion and EPN gave satisfactory control of all peach insects studied. Chlorthion and Guthion also gave satisfactory control of the oriental fruit moth and the plum curculio, but these materials were not tested for control of insects causing catfacing. In laboratory studies parathion, dieldrin, malathion, and Diazinon gave from 90% to 100% kill of first generation plum curculio adults within 5 days following treatment. Thimet and demeton gave unsatisfactory kill within a similar period.

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Further Tests with Insecticides to Control the Southwestern Corn Borer[1]

R. R. Walton,[2] K. D. Arbuthnot,[3] and J. S. Brooks[2]

This is the second report on insecticide test to control the southwestern corn borer (*Zeadiatraea grandiosella* Dyar) in Oklahoma. Results of tests made in 1953 were reported by Arbuthnot & Walton (1954). Rolston (1955) made a similar report on tests conducted in Arkansas.

In 1954 applications of insecticides with a fixed-boom sprayer on young corn against first-generation borers gave no control. Nine insecticides were compared in two applications of 0.5 pound toxicant in 35 gallons of water per acre. Granulated formulations of five insecticides applied twice with a rotary-type hand duster at 0.5 pound toxicant per acre were also ineffective.

Methods and Materials.—In view of the failure of power applications in 1954, tests in 1955 were designed to refine techniques, using hand equipment and single-plant plots. A total of seven tests were made with emulsions and granules in three separate experiments during July, August, and September. In two experiments the borer infestation was induced by manually applying eggs to the plants; the third test was made on a natural infestation. The effects of endrin spray applied to three different parts of the plant were compared. These included (a) top application (nozzle held at a point approximately 18 inches directly above plant), (b) ear-zone application (spray directed toward opposite sides of the plant to cover that approximate portion from a point 12 inches above the uppermost ear to a point 18 inches beneath the lowest ear), (c) entire plant application (a combination of the top and ear-zone applications). Endrin spray was tested at volumes of 10, 35, and 100 gallons per acre. The effect of moisture on granulated formulations was studied. Immediately following application of granules, 10 gallons of water per acre, simulating dew, and 100 gallons per acre, simulating light rain, were sprayed on plants and these treatments compared with the granulated material which had no added moisture. Endrin sprays were tested in one and two applications, starting at 5 and 8 days after oviposition began. Six toxicants were compared in spray applications at 35 gallons per acre. These included endrin, Phosdrin, Carbide & Carbon No. 7744 (Sevin)[4] and Thimet, Am. Cyanamid 12008 and 12009. In addition, in two of the tests, 2.5% endrin granules of 30–60 mesh particle size were applied. With the exception of the granulated treatments, all toxicants were applied at a rate of 1 pound per acre. Granules were applied with a rotary-type hand duster. Sprays were applied with a compressed-air sprayer and a 0.067 *Tee-jet* fan-type nozzle at 50 p.s.i. Treatments were replicated from 10 to 30 times in single-plant plots in randomized blocks. All tabulated data are presented as the average per 20 plants.

Table 1.—Results from endrin spray treatments applied to different parts of corn plants for control of the southwestern corn borer. 1955.

	Part of Plant Treated			Untreated Check
	Ear Zone	Entire Plant	Top	
Number of damaged plants	0.6	2.2	2.6	10.4
Number of borers	0.6	1.8	1.8	14.0

Table 2.—Comparative borer control resulting from different volumes of endrin spray. 1955.

	Gallons per Acre			Untreated Check
	10	35	100	
Number of damaged plants	4.0	2.0	0.4	10.4
Number of borers	2.4	1.2	0.0	14.0

Table 3.—Comparative borer control resulting from the application of different amounts of water to plants after treatment with endrin granules. 1955.

	Gallons per Acre			Untreated Check
	0	10	100	
Number of damaged plants	2.8	2.8	0.4	10.4
Number of borers	3.6	1.2	0.4	14.0

Table 4.—Comparative borer control resulting from one and two applications of endrin spray. 1955.

	No. of Applications		Untreated Check
	One	Two	
Number of damaged plants	1.7	0.0	10.9
Number of borers	1.4	0.0	15.2

Table 5.—Comparative effectiveness of seven toxicants applied in sprays August 17, 1955, against the southwestern corn borer.

Toxicant	Number of	
	Damaged Plants	Borers
Endrin	2.0	2.0
Am. Cyanmid 12008	4.0	5.3
Am. Cyanamid 12009	7.3	7.3
Phosdrin	8.7	10.7
C. & C. 7744	8.7	10.7
Thimet	9.3	12.0
Untreated check	11.3	16.0

Results.—Table 1 gives a summary of the results of tests comparing spray applications to different parts of the plant. Ear-zone, entire, and top applications were effective in the order listed. These results suggest that the

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[2] Oklahoma Agricultural Experiment Station.

[3] Entomology Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

[4] N-methyl-1-naphthyl carbamate.

insecticide should be concentrated on the ear-zone to give most efficient control.

The effect of volume of water on endrin spray is summarized in table 2. The results showed effectiveness generally increased with the volume of water up to 100 gallons per acre.

Water was tested also as a factor when applied following the application of granules (table 3). The results of these treatments suggested that light rains would increase the effectiveness of granules since very little borer injury occurred in the 100-gallon treatments.

Emulsion sprays and granulated formulations of endrin were compared in two experiments. These treatments were not completely comparable because the application rates of the granules were only 0.3 and 0.67 pound of toxicant per acre, well below the 1-pound level intended in order to equal that applied in spray treatments. The spray treatments averaged 2.1 damaged plants and 1.3 borers compared with 2.5 damaged plants and 2.2 borers for the granulated treatments. This indicated that at equal rates of application, granules would be as effective as sprays.

One and two applications of endrin spray were compared in five paired tests (table 4). All two-application treatments and one of the single applications gave complete control. The average for the five single applications was 2.0 damaged plants and 1.6 borers, well below the numbers found in the check.

Two time-of-treatment schedules were compared in four tests involving two applications per test. In the early schedule, the first application was made 5 days after eggs were first found or placed on plants and the second application applied one week later. In the later schedule, treatment was started on the eighth day after eggs were first found. The eighth-day schedule gave an average of 0.5 damaged plants and no borers, whereas the fifth-day schedule gave 1.5 damaged plants and 1.0 borer per test. Single applications made 5, 8, and 12 days after eggs were first recorded had 2, 4, and 1 damaged plants, respectively, and 2, 2, and 1 borers per test respectively. These results suggest that effective schedules may be delayed several days beyond the fifth day after oviposition begins.

Endrin was the most consistently effective toxicant tested in 1955. In a test on natural infestation which produced 11.3 damaged plants in the untreated check endrin gave 2.0 damaged plants compared with 4 to 9.3 damaged plants for six other materials (table 5). In two other tests where manually applied infestation produced from 10 to 18 damaged plants in the untreated checks there were no significant differences between toxicants and all of them gave very good control. In the first test under natural infestation hatching occurred over a period of several days while in the two manually infested tests hatching did not extend longer than a period of 4 days. Under this condition the greater residual effectiveness of endrin compared with the other materials was not reflected.

It is noteworthy that the borer populations in 1955 were very low; 57% infestation with an average of 0.8 borer per plant in untreated plots, whereas in 1953, all plants were infested with an average of 7.8 borers per plant (Arbuthnot & Walton 1954). The low population in 1955 resulted from the extreme drought in 1954, which caused nearly all corn to die by midsummer, thus greatly reducing the succeeding overwintering and emerging populations.

The abnormally low borer populations present in these tests prevent results from being conclusive. It is believed, however, that trends in effectiveness of the methods tested are indicated and that these are of value, particularly in view of the limited knowledge available on the control of this pest.

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Leafhoppers Associated with Forage Crops in Rhode Island[1]

T. W. KERR,[2] *University of Rhode Island, Kingston*

Alfalfa, red and Ladino clover, orchard grass, bromegrass, perennial ryegrass and timothy are commonly grown for pasture, hay or silage in Rhode Island. Beginning in 1948, a survey was undertaken to ascertain the insects of economic importance in the production of these crops. With the exception of 1950, it has continued each year with the crop emphasis changing from time to time. Among the insects collected were several species of leafhoppers. It is recognized that this group of insects is of economic importance, not only because of the depredations caused to the crops themselves, but because it contains many species that are proven carriers of plant diseases. This is an account of the leafhoppers collected and concerns (1) the relative abundance, (2) number of broods or peaks of population, (3) distribution throughout the season, and (4) the preferred host plants of the species found in greatest numbers.

METHODS.—Seasonally the leafhoppers were collected, along with other insects in the forage crop insect complex, by making 100 sweeps with a 12-inch collecting net in the various crop areas on the number of occasions indicated in table 1. Weather conditions permitting, the collections were begun each year during the period of mid-April to early May and were terminated during late October. The seven legume-grass associations were part of a study of pastures for Rhode Island; their seasonal botanical composition having been described by Stuckey (1952). They consisted of orchard grass-Ladino clover, reed canary grass with alfalfa and with Ladino clover,

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[2] The author gratefully acknowledges the assistance of Dr. D. A. Young in establishing the identity and taxonomic position of the leafhopper species mentioned herein.

Table 1.—The relative numbers of several species of leafhoppers collected from various forage crops in Rhode Island during the years indicated.

Year	Collections: Interval Between (Days)	Collections: Number During Season	*Macrosteles fascifrons*	*Empoasca fabae*	*Endria inimica*	*Draeculacephala antica*	*Aceratagallia sanguinolenta*	*Agallia constricta*	*Graminella nigrifrons*	Immature and Other Leafhoppers	Total
					Seven Legume-Grass Pastures						
1948	14	56	1355	142	366	151	17	11	525	252	2819
1949	13	84	665	80	1684	41	384	124	419	356	3753
	Per cent of total		30.7	3.4	31.2	2.9	6.1	2.1	14.4	9.3	
	Average per 100 sweeps		14.4	1.6	14.6	1.4	2.9	1.0	6.7	4.3	
					Bromegrass-Ladino Clover						
1948	14	8	161	20	42	19	10	2	46	11	311
1949	13	12	132	7	253	8	158	27	40	23	648
1951	12	15	665	33	68	11	48	32	110	20	987
	Per cent of total		49.2	3.1	18.7	2.0	11.1	3.1	10.1	2.8	
	Average per 100 sweeps		27.4	1.7	10.4	1.1	6.2	1.7	5.6	1.5	
					Red Clover						
1951	17	10	275	20	60	5	115	83	134	99	791
1952	8	10	638	49	51	6	132	192	74	181	1323
1953	11	14	94	41	30	0	37	297	92	29	620
1954	10	60	1002	144	28	34	80	267	59	42	1656
	Per cent of total		45.8	5.8	3.8	1.0	8.3	19.1	8.2	8.0	
	Average per 100 sweeps		21.4	2.7	1.8	.5	3.9	8.9	3.8	3.6	
					Alfalfa						
1953	12	11	19	369	1	6	1	31	3	68	498
1954	7	29	143	191	110	25	33	68	11	81	662
1955	10	47	491	63	65	28	27	62	62	13	811
1956	11	21	386	165	113	29	26	135	54	51	959
	Per cent of total		35.5	26.9	9.9	3.0	3.0	10.1	4.4	7.3	
	Average per 100 sweeps		9.6	7.3	2.7	0.8	0.8	2.7	1.2	2.0	
	Per cent of total from all crops		38.1	8.4	18.1	2.3	6.7	8.4	10.3	7.7	

perennial ryegrass with alfalfa and with Ladino clover, and colonial bentgrass with bird's-foot trefoil and with Ladino clover. All the above associations, except those having colonial bentgrass, contained a larger percentage of grass than legume. The fields of bromegrass-Ladino clover consisted of approximately equal parts of grass and clover. The red clover varieties encountered in the various fields were medium red, Kenland, Dollard and Pennscott; those of alfalfa were Atlantic, Grimm, Narragansett, Ranger and Vernal.

All crop fields, except those of alfalfa, were located either in Kingston or within a radius of 4 miles. The fields of alfalfa were in Kingston and at scattered locations throughout the state. Both volunteer grasses and other weeds were present in small quantities in all fields.

Results.—A total of 15,838 leafhoppers representing 14 species were collected in 37,700 sweeps. These included *Agallia constricta* Van D., *Aceratagallia sanguinolenta* (Prov.), *Draeculacephala antica* (Walk.), *Helochara communis* Fitch, *Dicranoneura loca* Del. & Cldw., *Empoasca fabae* (Harr.), *Cloanthanus frontalis* (Van D.), *Macrosteles fascifrons* (Stal.), *Macrosteles lepidus* (Van D.), *Paraphlepsius irroratus* (Say), *Deltocephalus balli* (Van D.), *Deltocephalus flavicosta* (Stal.), *Endria inimica* (Say), and *Graminella nigrifrons* (Forbes). Data on seven of the most numerous species are included under respective boxheads in table 1. "Immature and other leafhoppers" refers to undetermined specimens of immature leafhoppers and to adults of species listed above which were not numerous enough to warrant being included separately in table 1. In this category approximately one-third of the total number given were adults, the remainder being nymphs.

Macrosteles fascifrons was the most abundant species collected. In discussing this insect Bierne (1952) mentions three or more over-lapping broods. In Rhode Island, there was evidence of three broods, with the first peak of population in early to mid-June, the second during mid- to late July and the third in late August or early September. Adults were first collected in mid-May and were found in the field until late October. The insect was more prevalent on red clover and the bromegrass-Ladino clover pastures than in fields of alfalfa. Among the grasses it showed a preference for reed canary grass and was less abundant on orchard grass.

In accordance with the statement of Osborn (1939), *Endria inimica* was found to have two broods annually. Adults of the first brood began to appear by mid-June, the peak of population being reached by early July. A second population peak occurred in early September. Adults were found in diminishing number until late October. The species was less prevalent on alfalfa and red clover than on the various legume-grass associations where it showed prefernce for reed canary grass.

Although Osborn (1912) mentions that *Graminella nigrifrons* has two broods annually, the evidence obtained

in Rhode Island indicated three broods with peak populations occurring in late June, mid- to late August, and late September to mid-October. Adults were first collected in early June and were found in the field until late October. It showed a preference for red clover and the various legume-grass associations. In the latter category it showed a preference for orchard grass. Few specimens were obtained from alfalfa.

Adults of *Empoasca fabae* were encountered first either in late May or early June on forage crops. There was no distinct peak of population, the insect being taken in small but consistent numbers throughout the season. It showed a preference for alfalfa.

The evidence obtained relative to *Agallia constricta* shows two peaks of population, the first of which occurred either in late May or early June and the second in early to mid-August. Adults were found as early as May 1, but were not collected after late September. The insect was most prevalent on red clover.

Although *Aceratagallia sanguinolenta* was not abundant, the evidence indicated two broods annually, the peak population of the first being about mid-July and the second in late August or early September. Adults were first collected in late May and also were found late in October. The insect showed a preference for red clover and Ladino clover, but not for alfalfa.

Relatively few specimens of *Draeculacephala antica* were collected. They were obtained as early in the season as May 16 and were found as late as October 6. Although insufficient quantities were available to firmly establish the number of broods, there was an indication of two broods with peak adult populations occurring in early June and early to mid-August. The insect appeared to prefer grasses rather than legumes.

SUMMARY.—The leafhoppers associated with the production of alfalfa, red clover and several legume-grass associations were investigated in Rhode Island during the period of 1948 to 1956, exclusive of 1950. Fourteen species were collected, the most abundant of which were *Macrosteles fascifrons* (Stal.), *Endria inimica* (Say), *Graminella nigrifrons* (Forbes), *Agallia constricta* Van D., *Empoasca fabae* (Harr.), *Aceratagallia sanguinolenta* (Prov.), and *Draeculacephala antica* (Walk.). Data on the relative abundance, number of broods or peaks of population, distribution throughout the season and preferred host plants of the species found in greatest numbers are given.

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Aerial Applications of Granulated Dieldrin for Control of Larvae of the European Chafer on Hilly Meadows and Pastures

W. G. EVANS, H. H. NEUNZIG, and GEORGE G. GYRISCO, *Cornell University, Ithaca, New York*
and
ROY G. RICHMOND, *Plant Pest Control Branch, Agric. Res. Serv., U.S.D.A.*

In earlier papers, (Gyrisco & Evans 1954, Evans & Gyrisco 1955) the authors reported good results in controlling the grubs of the European chafer, *Amphimallon majalis* (Raz.), in pastures and meadows with the use of granulated insecticides applied by hand-operated equipment. Since most of the areas of larval infestations of the European chafer occur on the hilly glacial deposits known as drumlins in Central New York and are very difficult to treat rapidly with any powered machinery, it appeared highly desirable to determine whether these infestations could be treated successfully by the application of a granulated insecticide by airplane with a minimum of residue and drift to adjacent areas.

METHODS AND MATERIALS.—Four typical hilly areas were chosen for these experiments in and around Newark, (Wayne Co.) New York. These areas will be designated arbitrarily as the Jackson-Perkins Farm, the Dennis Farm, Hutchby-Cooper Farm A and Hutchby-Cooper Farm B.

The Jackson-Perkins Farm test site consisted of a 30-acre pasture which had been seeded in 1952 to alfalfa of the variety, Ranger, and orchard grass. It was situated on the western slope of a steep drumlin inaccessible to most farm machinery. The winds at this site were westerly at the time of insecticide application.

The Dennis Farm test site was a 25-acre pasture which had been seeded to orchard grass and timothy, but now consisted primarily of Canadian thistle, dock, plantain, mullein, dandelions, redtop, quack grass, and other obnoxious weeds. The test field was situated on the eastern side of a very steep drumlin on the leeward side of the prevailing winds.

The Hutchby-Cooper Farm A site was 30 acres in size and consisted of a 3-year-old stand of Ranger alfalfa situated over a broad drumlin running north and south. This area was somewhat level and accessible to standard farm machinery. Here, the wind was from the northwest. The second site on the Hutchby-Cooper Farm was a 7-acre area of slightly sloping land that was seeded to winter wheat.

It was decided that only a single toxicant, dieldrin, should be used, and that two proprietary granulated formulations of 5 and 10% dieldrin would be applied at 30 pounds per acre. The Jackson-Perkins pasture and the Hutchby-Cooper Field A were treated with the 10% formulation while the 5% formulation was applied to the

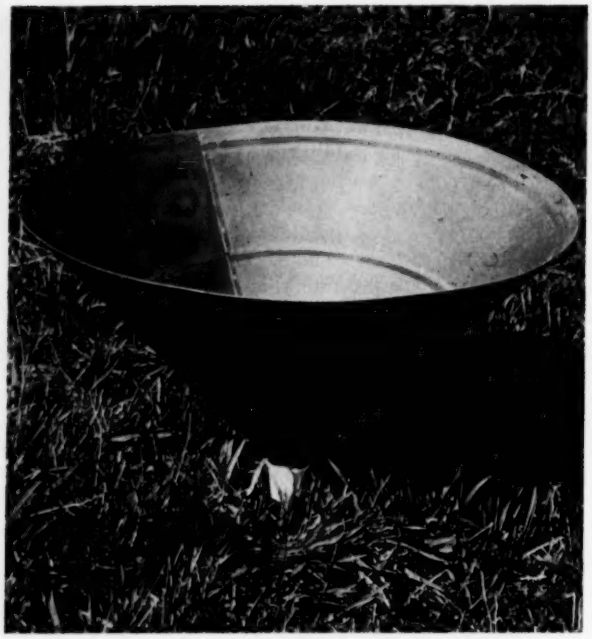

FIG. 1.—Aluminum cone mounted on a steel peg to catch insecticide deposits.

other two fields. The insecticides which were freshly formulated with Granular Attaclay were said to be of a 30/60 mesh size, although they were of poor consistency and varied in size from a fine dust to a few pellets as large as 0.5 inch in diameter.

Prior to application of the insecticides, random areas on all farms were sampled for grubs of the European chafer to determine the levels of infestation. These samples consisted of square-foot diggings to a depth of 9 inches, with the soil in each hand-sorted for the insects. (See Table 1.) In addition a member of the Department of Wildlife and Conservation at Cornell University made a field mouse survey using baited traps in the areas and noted the location of various streams near the test sites so that a check could be made on the influence of the insecticides on the existing wildlife.

Just before treatment commenced, large aluminum cones measuring a 0.5 square yard in area at their opening, and mounted on a steel peg, were set out in the test areas. These cones were so mounted that any insecticide

Table 1.—European chafer grubs found before and at two intervals after aerial application of granulated dieldrin. Newark, N. Y.

Location	Pounds Actual Toxicant per Acre[a]	Grubs per Square-Foot Sample[b]		
		Before Treatment	Sept. 27, 1954	Oct. 12, 1955
Jackson-Perkins Farm[c]	0.73–3.58	1.1	0	0
Dennis Farm[c]	0.87–2.76	6.0	0	0
Hutchby-Cooper Farm A	0.82–1.82	5.0	0	0
	none	—	5.5	6.1
Hutchby-Cooper Farm B	0.95–2.71	5.0	0	0
	none	—	2.3	7.0

[a] Applied April 20, 1954.
[b] Mean number of grubs found in 20 random samples each one square foot in area and approximately 9 inches deep.
[c] No suitable untreated areas nearby.

Table 2.—Weather conditions just prior to application and distance of drift of the insecticide at each location, April 20, 1954. Newark, N. Y.

Location	Average Wind Velocity (m.p.h.)		Distance of Drift in Yards[a]	Temperature (°F.)	Relative Humidity (Per Cent)
	Top of Drumlin	Bottom of Drumlin			
Jackson-Perkin Farm	15	4	>350 H	78	26
George Dennis Farm	10	3	>200 L	80	51
Hutchby-Cooper A	8	5	>300 M	75	69
Hutchby-Cooper B	—	—	>300 M	—	—

[a] H—heavy drift; L—light drift; M—moderate drift.

which was caught was immediately funneled into a small, labeled, paper cup. (See Fig. 1) All the cones or funnels at three locations were mounted at an arbitrary 5-foot interval. In this manner the actual deposit and pattern of deposit could be determined. At the Jackson-Perkins Farm the cones were mounted across the entire width of the experimental site at right angles to the flight of the aircraft, and in addition a few funnels were mounted at random in arbitrarily chosen areas. A similar design was used at the Dennis Farm except that the cones were spaced 10 feet apart and no randomly placed funnels were used. At the Hutchby-Cooper Farm A, the funnels were set up parallel to the direction of flight of the aircraft, and on Farm B they were mounted at an oblique angle of about 30 degrees in relation to the direction of flight of the aircraft.

In addition to using the metal cones to evaluate the insecticide deposit pattern, square-foot plates of glass, which had been covered with a thin layer of tanglefoot, were placed at random in three of the treated areas to determine the pattern of the distribution of the granules on the surface.

On April 20, 1954, all the areas were treated by means of a 450-horsepower Stearman, PT-17 equipped with a Transland Sprayer-Duster calibrated to apply the granulated insecticide at the rate of 30 pounds per acre. All fields except Hutchby-Cooper B were treated along the long axis, while Hutchby-Cooper B was treated up and down the slope of the field. The aircraft was flown at 100 m.p.h. at an altitude of 25 feet. Flaggers stood at the ends of each field to guide the pilot in applying the insecticide in approximately 100-foot swaths on each flight. At the time of each treatment, measurements were made of the temperature, humidity, wind velocity, and wind direction. Notes were also taken on the amount and distance of drift. These data are presented in table 2.

Immediately after treatment, the cups were collected from the cones and the contents were carefully weighed later in the laboratory where the deposits were calculated on a pounds-per-acre basis of actual toxicant. These data are given in table 3. The glass plates were also taken to the laboratory and, after marking off each plate into square inches, counts were made of the number of granules in five randomly selected areas on each plate. The results of these counts are shown in table 4.

On September 27, 1954, and again on October 12, 1955, the treated fields were sampled for larvae of the chafer by digging 20 one-square-foot holes to a depth of approximately 9 to 12 inches, and hand sorting the soil for the

Table 3.—Amounts of actual dieldrin applied by aerial application per acre as a granulated insecticide calculated from quantities of material caught by a series of 0.5-yard-square cones.[a] April 20, 1954. Newark, N. Y.

No. and Position of Cone	Jackson-Perkins Farm		Dennis Farm	Hutchby-Cooper Farm A	Hutchby-Cooper Farm B
	In Swath	At Random	In Swath	In Swath	In Swath
0	3.00C	3.00C	1.50C	1.50C	3.00C
1	4.07B	3.58	1.66	0.87	1.82
2	3.60B	1.48	1.95	0.83	0.95
3	2.02B	1.84	1.86	0.82	2.74
4	1.95	0.00	1.00	0.79	2.65
5	2.29	1.24	1.26	0.98	2.71
6	2.38M	2.09	2.76	1.72	2.22
7	2.02M	4.16	2.24	1.26	3.82
8	1.60M	1.50	1.38	1.21	3.20
9	2.33	0.00	1.01	1.82	2.41
10	1.80	0.73	0.87	1.06	2.59
11	1.05	0.94			3.13
12	0.41T	3.31			2.21
13	0.24T	3.26			1.46
14	0.14T				1.33
15	0.08T				0.22E
16	0.07T				0.42E
17	0.04T				0.68E
18	0.02T				0.02E
19	0.01T				0.02E
Mean	1.37	1.86	1.59	1.14	2.37
Median	1.05	1.50	1.52	1.09	2.50

[a] C—calculated, theoretical dosage; B—Bottom of drumlin; M—middle of drumlin; T—Top of drumlin; E—Hopper near empty, data not in mean.

insects. Whenever possible, adjacent untreated areas were also dug to serve as checks. (See Table 1.)

Nine days after treatment five one-square-foot forage samples of standing alfalfa on Hutchby-Cooper Farm A were clipped at cutterbar height, composited, subsampled, and stripped green for analysis to determine the amount of residue remaining on the foliage. (See Table 5.)

DISCUSSION.—As may be seen in table 1, excellent control of two generations of the larvae of the European chafer was obtained from aerial applications of approximately 1.5 and 3.0 pounds of actual dieldrin per acre, indicating that grub control can be achieved over difficult terrain by aerial applications of granulated insecticides. These results suggest that other soil insects could possibly be controlled by similar methods and formulations.

In studying the deposits from the Jackson-Perkins Farm experimental site, (See Tables 3 and 4), it is quite evident that the turbulence, caused by the wind passing over a hill, greatly affected the uniformity of deposit even though such wind was not of a great magnitude. As might be expected, the effects of the wind were greatest at the top of the hill. Some difficulty was also encountered in calibrating the dispersal mechanism and as a result only a few areas were covered with the desired dosage. The mean and median of all deposits were only about one-half of the amount desired. At the Dennis Farm where there was less wind and better protection of the experimental site by trees the deposit was more nearly uniform but still varied by nearly 150% between the lowest and the highest deposits. However, the mean and median deposits of insecticides were very close to the calibrated dosage of 1.5 pounds per acre. Again, on the

Table 4.—Number of particles of insecticide per square inch found on glass plates covered with tanglefoot placed in three fields treated by aircraft with 3 pounds of granulated dieldrin per acre. April 20, 1954. Newark, N. Y.

Plate No. and Square Inch		No. of Particles per Square Inch, 5 Square Inches per Plate		
		Jackson-Perkins Farm	Hutchby-Cooper A	Hutchby-Cooper B
1.	a	23	18	19
	b	42	20	22
	c	43	21	22
	d	44	24	23
	e	48	31	25
2.	a	42	48	85
	b	42	53	96
	c	43	64	99
	d	44	69	101
	e	51	84	107
3.	a	89		
	b	90		
	c	93		
	d	104		
	e	117		
4.	a	61		
	b	63		
	c	66		
	d	69		
	e	72		
5.	a	34		
	b	42		
	c	43		
	d	49		
	e	53		
Mean		58.68	43.2	59.9

Hutchby-Cooper Farms the dosage did not equal that which was desired and the deposits were highly irregular across the breadth of each swath and the whole experimental site.

The residues of dieldrin remaining on the foliage (from 0.4 to 0.9 p.p.m.) are higher than those reported by Evans & Gyrisco (1955) for dieldrin applied at the rate of 2 pounds of actual toxicant per acre by hand-operated equipment, where the highest residue was 0.4 p.p.m., but it is believed that these larger amounts of residues were

Table 5.—Residue[a] from dieldrin granules remaining on foliage of alfalfa 9 days after treatment by airplane. Hutchby-Cooper Farm A. April 29, 1954. Newark, N. Y.

Actual Dieldrin in Pounds/A. (Calculated)	Residue Found[b] (p. p. m.)
1.5	0.42
1.5	.64
1.5	.78
1.5	.80
1.5	.90
None	.17

[a] The assistance of Shell Chemical Corporation in analysing these samples is gratefully acknowledged.

[b] ANALYTICAL METHOD: Extraction—Hexane—1 PA Extraction; Cleanup—Chromatography with MgO; Analysis—Reduction—Phenyl azide method; Sensitivity: 0.17 p.p.m. ANALYSIS OF RECOVERY SAMPLES: at 0.1 p.p.m.—120%; at 0.3 p.p.m.—86 %.

due to the greater proportion of fine materials in the formulation resulting from breakage of granules during shipment. The samples, however, were taken only 9 days after treatment, and there is no question that growth of foliage and degradation of the insecticide would have cut the residues to a very low level by harvest time nearly three weeks later. In general, it would be best to treat such meadows in late fall or early in spring prior to the new growth of the season to lessen the chances for excessive residues.

A study of the field mouse population before and after the treating of the field showed no change which could be attributed to the application of insecticides. None of the nearby streams showed any dead fish resulting from insecticide drift which was noted for over 300 yards in some cases. Dead earthworms were found along a drainage ditch in one field immediately after treatment, but on sampling the field for European chafer grubs 6 months later, there appeared to be no fewer earthworms in the treated than in the untreated soil.

SUMMARY.—Four large hilly areas were treated by aircraft with approximately 1.5 and 3.0 pounds per acre of actual dieldrin which was applied as a granulated formulation with Attaclay for the control of the larvae of the European chafer. Excellent control was achieved for the two generations of grubs during which the experiment was in progress.

Measurements of the uniformity and amount of insecticide deposits by the use of special aluminum cones and tanglefoot coated plates showed that the deposits were irregular and in most cases did not approach the amounts desired. Air currents greatly affected the deposits over the hilly terrain.

Amounts of insecticide residue on the forage were determined 9 days after treatment, and showed the residues to be higher than expected in comparison with previous tests. This was, in part, attributed to the poor consistency of the formulation, brought about by breakage of the granules during shipment.

A survey of wild life in the area treated, before and after treatment, showed no appreciable change in the field mouse population nor any toxicity to fish from drift. Drift was greater than expected extending over 300 yards. Some earthworms were killed immediately after treatment, but no reduction in population was noted after a few months.

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Recent Developments in the Plant Pest Control Branch[1]

E. D. BURGESS, *Chief, Plant Pest Control Branch, Agric. Res. Serv., U.S.D.A.*

Doubtless you are all aware that activity in the field of pest control relating to insects, plant diseases, and nematodes has increased considerably in tempo during the past few years. Part of this, particularly that pertaining to pests of foreign origin, can undoubtedly be attributed in great measure to the recent unprecedented advancements in the means for and the volume of trade in commodities of one kind or another from foreign countries and in passenger travel. While it will be impossible to discuss fully how this acceleration has affected those of us in State and Federal control and domestic regulatory work, I would like to point out some of the things that are of intense interest to us at the moment. First of all, however, I should comment briefly on recent changes with regard to our organizational structure in order that you will understand our present mode of operations.

CHANGES IN ORGANIZATIONAL STRUCTURE.—Many of you will recall that before the Plant Pest Control Branch of the Agricultural Research Service was split out of the Bureau of Entomology and Plant Quarantine steps were taken to regionalize its activities, especially those dealing with domestic regulatory work. Five regions were then established with a Regional Director in each who had jurisdiction over all of the projects which were headquartered within the geographical boundaries of his Region. While this led to a partial integration of forces and combined all of the business activities of the old Bureau, projects overlapped and certain inherent difficulties soon became evident. Recognition of its shortcomings led to a rather comprehensive study of the good and bad points of the system, the results of which we are now implementing. The former project system operated wherever the problems dictated throughout the country. Our new organization adheres closely to geographical limits.

We have used the boundaries of the State Plant Boards to denote our four Plant Pest Control regions. To these we have added a fifth, one in the Republic of Mexico, where we co-operate with the Mexicans on problems of particular concern to the southern United States. Regional headquarters for the four domestic regions have been established near the business offices of the Agricultural Research Service. They are located as follows: the Eastern Region in Moorestown, New Jersey, a suburb of Philadelphia; the Central Region in Minneapolis, Minnesota; the Western Region in metropolitan San Francisco, California; and the Southern Region in Gulfport, Mississippi. The Region in Mexico is located in Monterrey, Nuevo Leon.

Each region has responsibility for all assigned co-opera-

[1] Invitation paper presented at the Twenty-eighth Annual Meeting of the Eastern Branch of the Entomological Society of America held in Atlantic City, New Jersey, November 19–20, 1956. Accepted for publication December 12, 1956.

tive plant pest control and domestic regulatory programs falling within it. The Regional Supervisor of the Eastern Region, for example, is not only responsible for carrying out all phases of the gypsy moth program in his region but also those functions pertaining to the Japanese beetle, the European chafer, barberry eradication, the golden nematode, or other problems that may be undertaken as time goes on. While this structure automatically abolishes the projects, the changes are neither as sweeping nor as new as it might appear on the surface. You may remember that many years ago the Chief of the Bureau of Entomology and Plant Quarantine saw fit to integrate the regulatory work pertaining to the gypsy moth and Japanese beetle so that one inspector could certify products as free of both of these pests. The saving of time, effort, and money was obvious and the nurserymen involved were not subject to dual certification of their stock by inspectors from two different units of the same organization. The present regional structure will permit a similar multiple use of our inspectors throughout the Branch.

To carry out the work below the regional level we have established 34 area offices in the four domestic regions and two in the Republic of Mexico. Each Area Supervisor is assigned one or more states depending on the workload and the responsibilities involved. Each will have responsibilities across the board for the programs in his area in this manner carrying out the broad assignment of the Regional Supervisor.

This system extends beyond the Areas into Districts, the smallest geographical division in the structure. Each District Supervisor will be assigned one or more District Inspectors.

Experience in the southeast and in Michigan which were the proving grounds for this new type of operation, leads us to believe that a more efficient use of personnel and equipment will result. We anticipate that the expenses involved in long-distance travel will be considerably reduced making the savings available for more productive work in the field.

In addition, there appears to be a distinct advantage in having the field personnel work on several programs within an assigned area. In the first place it permits him to know his area of responsibility intimately. It makes the worker a more versatile employee and permits shifting in duties as the occasion or situation warrants. We will also have readily available a nucleus of trained workers around which we can rapidly expand or change emphasis in our programs as the situation demands.

The question often arises as to how similar programs can be co-ordinated between the various regions. Five units, called Sections, at our national headquarters have that responsibility. The Pesticide Regulation Section deals with the administration of the Federal Insecticide, Fungicide, and Rodenticide Act together with the responsibilities of the Department in the more recent Miller amendment of the Food, Drug, and Cosmetic Act with which you are all familiar. As most of its activities are centered in the Washington-Beltsville area, it has not been involved in this adjustment. The Plant Pest Survey Section formerly called the Economic Insect Survey Section, is assigned the duty of co-ordinating all survey information, in addition to co-operating with the states in the reporting of insect pest abundance data. The Methods Improvement Section will continue to be concerned with improving plant pest control and treatment methods needed in our work throughout the States. The Cooperative Control Operations Section will be responsible for the control or eradication functions of our programs wherever they occur. In a similar manner the Regulatory Operations Section will co-ordinate the quarantine phases of Branch activities and work towards the standardization of regulations—wherever possible throughout the country. Through this device we are assuring ourselves of a completely correlated operation.

PROGRAM ACTIVITIES.—Not only in the field of management are we experiencing the "new look" but in many of our program activities as well where a noticeable change of emphasis has taken place. We seem to have taken the old word "eradication" from the shelf, dusted it off, and placed it in a position of prominence as a goal to shoot at. This has not happened overnight but has been a gradual evolution linked hand in hand with the many new chemicals and improved practices that seem to make such an objective possible. You may be interested in some of the progress along these lines. Taking a quick look at programs in which the Branch is currently involved, we find that eradication has a place in many of them—the khapra beetle; the Mediterranean fruit fly; the Hall scale in California; the problem of eradicating incipient infestations of the citrus blackfly in northeastern Mexico and in the Valley of Texas; the eradication of the Mexican fruit fly in southern California and northwestern Mexico where it has not yet established a firm foothold; the recent effort aimed at the eradication of the gypsy moth; and the intriguing possibility of eradicating the golden nematode from Long Island if research now in progress so indicates, together with promising experiences on control of the white-fringed beetle. All number among those were the odds seem to favor such an objective.

Khapra beetle.—In the 2 years since the khapra beetle program was started state and Federal inspectors have devoted a lot of time in survey work to determine its range. About 59,000 inspections have been made in 34 states, and in Mexico. This backbending, tedious search has now given a pretty reliable picture as to what its distribution was in North America. Its relatively limited occurrence and the steady decline in numbers of infestations now showing up as a result of the treatment and regulatory program, give good reason to believe that our co-operative effort can and will be successful. We were fortunate in finding a fumigation method which virtually assures absolute cleanup of infested storage facilities. As of the end of October this year 370 of the 447 sites found infested have been so treated. We can report that by early next year our fumigations should be about current with the infestations of the khapra beetle now known. So far this has meant treating over 109 million cubic feet of infested storage space of the total of 140 million found infested in Arizona, California, New Mexico, and in the Republic of Mexico, freeing them of the beetle. On the basis of present knowledge 77 sites or about 31 million cubic feet of storage remain and contracts have been let for much of that.

Mediterranean fruit fly.—For the second time in a little over a quarter of a century, we are in the midst of eradicating the Mediterranean fruit fly from Florida. Results so far are most encouraging, particularly when it is realized

that only a few days over 7 months have elapsed since its initial discovery in Miami on Friday the 13th day of April this year. Three months have now passed since a new county has been added to the list of the 27 infested. This has particular significance because we have been able to increase the number of traps 10 fold since midsummer to a total of about 40,000—spotted from the Florida Keys to the northernmost limits of the citrus growing regions near the Georgia line. Traps are being operated in Alabama, California, Georgia, Louisiana, Mississippi, and Texas. The number of new infestations and extensions or recurrences of old ones are steadily decreasing. In several counties they have disappeared entirely. No flies have been recovered outside of Florida. By mid-October 725,000 acres—roughly an area the size of the State of Rhode Island—had been treated one or more times by bait sprays applied by aircraft, bringing the aggregate number of acres covered to about 5 million. This is equivalent to an area slightly greater than the land area of Massachusetts. Were a similar acreage to be sprayed in the District of Columbia it would have meant that its 61 square miles would have been covered 131 times. Only 158,000 acres are still under treatment, a reduction of 78% of the original spray area.

While the 10 million dollars allotted to the co-operative program thus far seems like a huge amount of money, it appears to be a good investment compared with the annual estimated cost to the growers of living with the pest, and the continuing costs of keeping it within bounds were eradication not possible.

Other programs.—Time will not permit more than mere mention of some other programs that are, or seem to be pointing towards the possibility of eradication, at least in part.

One of the long-standing eradication programs concerns Hall scale in California, a serious pest of stone fruits. Since 1941 it has defied our best efforts to eradicate it from its adopted home in two counties in north-central California. Evidence would seem to indicate, however, that sucess is near. This is being accomplished by a series of three consecutive annual fumigations of dormant host trees with HCN or the removal and destruction of them wherever possible. Altogether about 50,000 host plants have been involved. We now expect that, unless some unanticipated extension of infestation occurs, the last affected tree will be fumigated by the winter of 1957 and except for continued vigilance for further occurrences, the program will come to a successful conclusion.

In 1954 the Mexican fruit fly was found for the first time in northwestern Mexico and southern California near the international boundary. These trap captures have undoubtedly resulted from periodic introductions into Baja California through the increased passenger and commercial traffic from interior Mexico where the fly is common. As you may know, eradication is impossible in the Rio Grande Valley of Texas where it appears annually because of the ever present reservoirs of infestation in wild hosts in nearby Mexico which breed sufficient flies to permit a natural migration into the United States. In the far west, however, it is a different story. No such reservoirs exist and our program, therefore, is one of cooperating with Mexican regulatory officials to prevent infiltration from the south and east together with a continuous spray program in cooperation with Mexico and California at points where trap catches have occurred from time to time. At San Ysidro, California, on the Mexican border, three flies were caught during the season of 1956—the first since 1954—and only 14 flies in Baja California in northern Mexico. We believe that none of these infestations are established. The success of the program will be contingent on our ability to maintain a fly-free zone in northwestern Mexico, thus eliminating the constant threat of fly infiltration into citrus areas of the far western states.

Recent research information furnished us by both State and Federal research workers together with our own experience on some of our other important problems has been such as to give some encouragement that eradication may be considered in the future elsewhere. The development of soil insecticides that can be applied to the surface without the need for mechanical incorporation into the soil gives promise as a means of eradicating the white-fringed beetle from *known* infested areas. Except for a small infestation in New Jersey this serious agricultural pest has been found on some 500,000 acres scattered within eight southeastern states. Each year we have been able to whittle away at these infestations and have been very successful in reducing their size and intensity to a point where beetles cannot be found on 34% of them. However, as in other programs, the means at our disposal for detecting low populations is a serious limiting factor to ultimate success. Adequate survey methods is one important phase of our work that needs particular research attention.

The golden nematode is yet another where preliminary operational research with soil fumigants seems to give some promise that before too long it may be possible, once again, to safely propagate potatoes or tomatoes on lands on Long Island which have been taken out of production due to this serious pest. At the present time only about 4,000 acres needed for agriculture are involved, a relatively small amount if means eventually become available to eliminate infestation thereon. Even with exhorbitant costs of treatment, such would be far less expensive in the long run than the continuing expense of regulatory and associated activities throughout the years to come, or until such time as it is no longer possible to hold the nematode within reasonable bounds and greater and greater sections of the country will of necessity have to contribute to the prohibitive cost of raising potatoes in the presence of this pest.

As indicated earlier, time would permit only a rather sketchy outline of the approaches to a few of the problems facing us and our state co-operators today. Mr. O'Dell will talk to you about another, the gypsy moth problem, later on this program. We like to feel that as many of our efforts as possible be pointed towards the ultimate goal of eradication even though we realize, probably better than most, the many pitfalls, uncertainties, and possible disappointments that lie in the path of undertakings as complicated as these of selectively wiping out a nucleus of living organisms from a limited geographical area. We strongly urge you in research to aid us wherever and whenever you can through the further development of materials and procedures which will be helpful in simplifying the many tasks facing us to which we are dedicated.

The Salt-Marsh Caterpillar and Its Control in Arizona

W. A. Stevenson, William Kaufman, and Louis W. Sheets, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

The salt-marsh caterpillar, *Estigmene acrea* (Drury), was not considered a major cotton pest in Arizona prior to 1948, although plants at times were defoliated late in the season by this insect. This defoliation was thought to cause negligible damage. In fact it was considered by many to be beneficial, causing the cotton to open more rapidly with less boll rot and also making the fields more attractive to cotton pickers.

During the fall of 1948 the caterpillars bred up in cotton in the Salt River Valley to millions in numbers, causing one of the worst infestations in the history of the Valley. Unquestionably some damage occurred, especially in the late-planted fields, and some control measures would have been undertaken if an efficient and economical insecticide could have been recommended. The caterpillars were allowed to run their natural course, and as a result, after defoliating the cotton, they migrated to other high-value crops, particularly lettuce and sugar beets. To prevent serious damage to these crops, the farmers as a last resort dug trenches around the fields and ran water in them. Some even floated a light oil in the water.

Cage Tests.—As soon as it became evident that such an infestation was developing, cage tests with various insecticides available at the time were started on the University of Arizona's Experimental Farm near Mesa. A few preliminary tests conducted in 1947 had shown 20% toxaphene to be more promising than several of the other new insecticides, including 5% DDT, 5% DDT plus BHC (2% gamma isomer) and also 2% and 3% parathion. In the 1948 tests screen cages 3×3×3 feet were placed over cotton plants and 10 mature caterpillars introduced into each. The plants were then dusted with a plunger-type hand gun at the rate of 20 to 25 pounds per acre. All the dusts contained sulfur. The insecticides tested and the results are summarized in table 1. The highest kills were obtained with the mixtures that contained 15% of toxaphene plus DDT, BHC, or chlordane. These results were further substantiated by a small number of tests later in the season.

Field Tests with Toxaphene-DDT Mixture.—Since the toxaphene-DDT-sulfur mixture gave the highest kill in the cage test, a few field tests were inaugurated with it in late October 1948. Small plots in several heavily infested cotton fields were dusted with rotary hand guns at the approximate rate of 20 pounds per acre. The percentage of sulfur was reduced from 52.5 to 40%, as this gave the finished product a better dusting quality. In every test thousands of dead caterpillars of all sizes could be found on the ground after 24 hours, with few remaining on the plants. In the untreated areas they were still extremely numerous and active. Highly satisfactory results were also obtained in a number of fields of sugar beets being grown for seed that were treated with the same mixture, both by ground equipment and airplane. The caterpillars ceased feeding soon after coming in contact with the toxaphene-DDT mixture, although the maximum mortality came hours later.

Table 1.—Cage tests with combinations of sulfur with toxaphene, DDT, BHC, and chlordane for the control of salt-marsh caterpillars, Salt River Valley, Arizona, 1948.

Insecticide	Number of Caterpillars Per Test	Per Cent Mortality in 96 Hours
Toxaphene—		
15%+DDT 5%+sulfur 52.5%	40	97
15%+BHC (gamma isomer 1%)+sulfur 25%	20	94
15%+chlordane 5%+sulfur 37.5%	30	93
5%+BHC (gamma isomer 3%)+sulfur 25%	30	86
10%+BHC (gamma isomer 2%)+sulfur 25%	20	68
15%+sulfur 37.5%	10	50
20%+sulfur 50%	10	30
10%+DDT 10%+sulfur 30%	10	20
Chlordane—		
10%+sulfur 50%	10	20
10%+DDT 5%+sulfur 62.5%	10	10
DDT 5%+BHC (gamma isomer 3%)+sulfur 37.5%	10	20

Table 2.—Cage tests with Dilan and toxaphene-DDT for the control of salt-marsh caterpillars, Salt River Valley, Arizona, 1950.

Insecticide[a]	Number Dead After (Hours)			
	16	24	48	64
Untreated	0	1	1	1
DDT 5%+toxaphene 15%+sulfur 40%	13	23	28	30
Dilan 3%	8	19	29	30

[a] Thirty caterpillars used in each treatment.

Late in 1949 and 1950 a number of large-scale demonstrations were made in cooperation with several cotton growers and insecticide dealers. A dust containing 15% of toxaphene, 5% of DDT, and 40% of sulfur was applied by airplane at rates of 20 to 25 pounds per acre. Excellent results were obtained, the control being above 95%. Sprays depositing 3 pounds of toxaphene plus 1 pound of DDT when applied at the rate of 7½ gallons of the emulsion per acre showed equally as good control as the dusts. Three pounds of toxaphene alone in sprays was inferior to the combination of toxaphene and DDT.

These results were so outstanding that the toxaphene-DDT mixture was recommended for general use on cotton. It was also proved effective on many other cotton-insect pests as well as pests of other crops and is now widely used in many areas. When the caterpillars are allowed to build up to large numbers in cotton, defoliate the plants, and start migrating to other crops, they are extremely hard to keep under control with insecticides. Aluminum-foil barriers placed around the fields will protect them from the migrating caterpillars.

Resistance to Toxaphene-DDT.—The toxaphene-DDT mixture continued to give highly satisfactory results until the fall of 1954, when reports of poor control in the western part of the Salt River Valley were received. Higher dosages were tried, but still the results were unsatisfactory. Large numbers of caterpillars continued to be killed, but sufficient were left to cause economic damage. In fact, on a considerable acreage losses as high as ½ bale per acre were caused by the feeding of these caterpillars.

As a last resort high dosages of parathion were tried.

It was found that from ¾ to 1 pound of the technical material in a spray gave practically 100% control. Large acreages were treated with this insecticide, but owing to the hazards involved it is not recommended for general use.

In 1955 the toxaphene-DDT mixture again failed to give the desired control, not only in the western part of the Salt River Valley but in areas contiguous to it, and again parathion was used. The failures at least indicated a definite buildup of resistance to the toxaphene-DDT mixture. The suspicion of resistance was supported by laboratory tests conducted at College Station, Tex., where larvae from Arizona were compared with larvae from a local culture. Four times the poundage was required to obtain the same mortality of the Arizona as the Texas caterpillars.

TESTS WITH DILAN.—In screening tests conducted at College Station in 1949 Dilan had shown promise for the control of salt-marsh caterpillars. During the fall of 1950 this insecticide was tested in comparison with toxaphene-DDT in Arizona. In cage tests full-grown caterpillars were encaged over cotton plants, which were then dusted at an estimated rate of 20 to 25 pounds of a 3% dust per acre. The results given in table 2 show that a high kill was obtained in 48 hours. In small-scale field tests 3% Dilan at 20 pounds per acre also gave excellent control. Counts made at random before treatment showed an average of 15.2 caterpillars per plant, and 24 hours after treatment only 0.8 per plant.

Although Dilan was found to be satisfactory, since the toxaphene-DDT mixture was still giving the desired results, Dilan was temporarily sidetracked, as it was not readily available. Since the caterpillars had shown a resistance to toxaphene-DDT in 1954 and 1955, Dilan was again tried in late 1955. These tests were conducted in co-operation with the growers and insecticide dealers. The Dilan used was an emulsifiable concentrate containing 5 pounds of technical material per gallon. In the first trials 0.625 and 1.25 pounds of technical Dilan were deposited per acre. Both these dosages gave good control, the higher one killing more than 95% of the caterpillars present. A large acreage was then treated with approximately 1 pound per acre, and close observations revealed no failures.

Some Dilan was sold to the cotton growers for salt-marsh caterpillar control. It was equally as good as toxaphene-DDT was prior to the development of apparent resistance, but proved to be slower acting Generally 5 days were required to obtain maximum mortality. Cotton leaves brought into the laboratory at intervals up to 10 days after being treated continued to show a very high mortality of grown worms released and feeding on them.

BIOLOGICAL CONTROL.—Two dipterous parasites were found of considerable importance in controlling these caterpillars. They were determined as *Exorista larvarum* (L.) and *Rileymyia adusta* (Lw.). Several other species were of less importance. Since these parasites are susceptible to many of the insecticides generally used in cotton-insect control, their use no doubt accounts largely for the increase in caterpillar infestations.

EMERGENCE FROM PUPATION.—To determine the time of emergence from pupation, early in the winter of 1950 three hundred larvae were collected and brought into the laboratory and the emerging dates noted. The following numbers emerged each month: 5 in January, none in February or March, 100 in April, 61 in May, 21 in June, 47 in July, and 5 each in August and September, or a total of 244. The remaining 56 were either parasitized or died from other causes.

Growth and Fruiting Properties and Carbohydrate, Nitrogen and Phosphorus Levels of Cotton Plants as Influenced by Thimet[1]

JOSEPH HACSKAYLO, *Field Crops Research and Entomology Research Branch, Agric. Res. Serv., U.S.D.A.*[2]

Thimet [0,0-diethyl S-(ethylthio) methyl phosphorodithioate] when applied to cotton seeds as a carbon impregnated dust has been found to be an excellent systemic insecticide for the control of some insects up to 6 weeks following germination. A brief summary of results obtained from numerous field experiments performed throughout the cotton belt with this compound has been compiled by the American Cyanamid Company (1956). Although most workers agree on its insecticidal effectiveness, conflicting results have been observed concerning its influence on plant growth and development. Generally, Thimet caused reductions in seedling emergence and, occasionally, in yields of seed cotton. Since variations in the environmental conditions between test locations in the above mentioned work were extreme in some instances, conflicting results on plant responses would be expected.

The present study was initiated to determine some of the physiological effects of Thimet on the cotton plant when near optimum conditions for growth were maintained. In addition, it was hoped that the information resulting from the present study might throw some light on the problem of phytotoxicity.

MATERIALS AND METHODS.—Cotton seeds of the Empire variety were germinated in the greenhouse in tall 4-gallon glazed earthenware jars containing washed river sand. On November 1, 1955, one week following germination, the jars were flushed with 4 liters of a complete nutrient solution (Hacskaylo & Ergle 1955), and the seedlings thinned to five per jar. Subsequently, at weekly intervals, the nutrients were renewed by flushing each jar with 4 liters of fresh solution. Water was added when needed to maintain an adequate moisture level.

Ten days after germination 200 ml. of aqueous emulsions containing 0, 0.2, 2.0 or 10 gm. of technical Thimet

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[2] In co-operation with the Texas Agricultural Experiment Station.

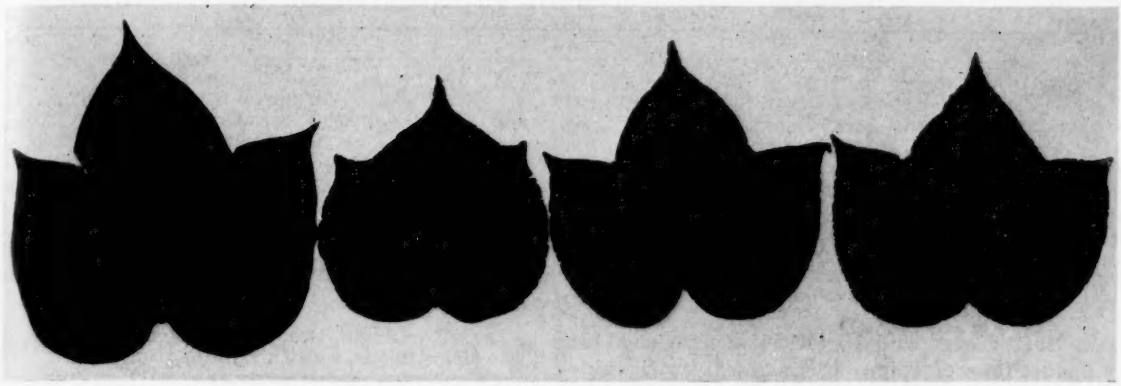

FIG. 1.—Interveinal flecking (white spots) in mature leaves of cotton plants growing in sand cultures of varying concentrations of Thimet. Left to right, 0, 10, 100 and 500 p.p.m.

per liter, emulsified with 0.1% Triton X-100, was added to each jar. These Thimet additions gave concentrations of 0, 10, 100 and 500 p.p.m. in the solution phase of the substrate. Each treatment consisted of five jars or 25 plants. One week after the addition of the Thimet emulsions, 4 plants were harvested from 3 replicates of each treatment. The root systems were thoroughly washed in de-ionized water, fresh weights determined and the plants immediately dried in a forced draft oven at 80° C. Four additional plants per treatment were harvested from the remaining two replicates and only the leaves retained and oven dried. The remaining plants (one per jar) were grown to maturity to furnish information on the effects of Thimet on growth, fruiting and boll properties. During the experiment, the flowers of these plants were tagged on the day of opening and the time of boll dehiscence was recorded. Boll shedding percentages were calculated from counts on the number of flowers produced and of bolls retained. Various growth and fruiting characters were determined 129 days following germination.

ANALYTICAL METHODS.—Plant material which had been dried for 24 hours was ground in a Wiley mill through an 80-mesh screen and stored in stoppered bottles. Soluble nitrogen was extracted from the dried plant material with water at 80° C. and determined by a semi-micro Kjeldahl method. Insoluble, or protein nitrogen in the residue was determined by the same method. Phosphorus was determined by methods as outlined in the Association of Official Agricultural Chemists (1935). Fiber analyses were made by the Cotton Ginning Research Laboratory, Mesilla Park, New Mexico.

Table 1.—Influence of Thimet on dry weight, boll shedding and yield of seed cotton.[a]

THIMET LEVEL IN SUBSTRATE (P.P.M.)	DRY WEIGHT PER PLANT (GMS.)	FLOWERS PRODUCED (NO.)	BOLLS RETAINED (NO.)	BOLLS SHED PER CENT	YIELD OF SEED COTTON (GMS.)
0	37.2	48	17	64.6	81.7
10	39.6	46	16	65.2	78.4
100	28.1	55	18	67.3	77.4
500	10.3	30	7	76.7	21.5

[a] Results based on five plants per treatment.

RESULTS.—*Growth and Phytotoxicity.*—Approximately 3 hours after the addition of Thimet, all of the plants receiving the insecticide had initially wilted; however, 24 hours later, all except those in the 100 and 500 p.p.m. levels had regained turgor. All levels of the insecticide caused marginal leaf burning by the fifth day which rapidly progressed to interveinal necrotic flecking by the end of the week. Cotyledon and leaf abscission occurred on plants receiving the 100 and 500 p.p.m. rates, with the latter being more severe. As growth continued, necrosis progressed to leaves developed at the higher nodes on the main stem. The incidence of flecking was correlated with Thimet concentration (fig. 1). Figure 1 also illustrates the amount of flecking found on the leaves of a mature plant treated with a high rate of Thimet (e.g. 500 p.p.m.). The terminal leaves would appear as the 0 concentration and the lowermost ones as the 500 p.p.m. level. Leaves produced above the fourth main stem node on plants grown in the 10 p.p.m. level were completely free of necrotic flecking, while those in the 100 p.p.m. series did not outgrow these symptoms until the 10-node stage. The plants on the highest level of the insecticide did not completely recover during the course of this experiment. The delay in growth of all of the treated plants was obvious up to the seventh week (fig. 2), at which time those receiving 10 p.p.m. were approximately equal to the controls, while those receiving the two highest levels were still inhibited. By the end of the experiment, the dry weights of plants treated with 10 p.p.m. of the insecticide had increased 6.5% above that of the controls, while those treated with 100 and 500 p.p.m. decreased 24.5 and 72.3%, respectively (table 1).

Fruiting and Boll Properties.—The lowest concentration of Thimet used (10 p.p.m.) had little effect on the number of flowers produced or the number of bolls retained as compared with the controls (table 1). Flower production of plants treated with the 100 p.p.m. concentration increased 14.6% more than the controls and continued for a longer period of time as compared with the other treatments. Six flowers per plant were produced on the highest level of Thimet (500 p.p.m.) of which only 1.4 were retained as bolls (table 1). The percentage boll shed was approximately the same for the controls, 10 p.p.m., and 100 p.p.m. plants; however, a 19.8% increase was found at the

Table 2.—Influence of Thimet on boll and seed properties.

Thimet Level in Substrate (P.P.M.)	Seed Cotton per Boll (Gms.)	Seeds per Boll (No.)	Weight of 100 Seeds (Gms.)	Lint on 100 Seeds (Gms.)	Weight of 50 Seed Coats (Gms.)	Weight of 50 Embryos (Gms.)	Ratio Embryo Weight per Seed Coat Weight (Gms.)	Interval for Boll Opening (Days)
0	4.81	26.3	12.1	6.1	2.90	2.78	0.959	49.9
10	4.90	26.4	12.4	6.2	3.03	3.06	1.009	49.9
100	4.30	29.3	13.6	6.6	3.10	4.13	1.330	52.0
500	3.60	19.7	13.0	5.3	2.80	3.80	1.360	48.9

highest level. The reductions in yield of seed cotton at the two highest rates of Thimet (100 and 500 p.p.m.) were caused by a reduction in boll size as measured by the weight of seed cotton per boll (table 2). A trend toward fewer seeds per boll and an increase in seed index was found as the concentration of the insecticide was increased in the substrate (table 2). Compared with the control and 10 p.p.m. plants, boll maturation was delayed 2 days on those receiving 100 p.p.m. of the insecticide, while a delay of 1 day occurred in those on the highest treatment level (500 p.p.m.). An increase in the ratio of embryo weight to seed coat weight was positively correlated with the level of Thimet in the substrate as larger embryos were produced in seeds of plants treated with the insecticide. Although Thimet had little effect on fiber length except at the 10 p.p.m. rate, all plants receiving the insecticide had coarser fibers (table 3).

Chemical Composition.—When expressed as percentage of dry weight, the reducing sugars, sucrose and starch contents of 20-day-old plants were directly correlated with levels of Thimet in the substrate (table 4). The carbohydrates were inversely correlated with dry weight production, indicating non-utilization of these reserves. Table 4 also shows that both soluble and protein nitrogen decreased in young plants, while the chloroform soluble and chloroform insoluble phsphorus levels in the leaves increased as the insecticide concentration was increased.

Table 3.—Length, strength and fineness of cotton fibers from plants treated with Thimet.

Thimet Level in Substrate (P.P.M.)	Length (in.)		Tensil Strength (100 lb./sq. in.)	Fiber Weight per Inch (μgms.)
	Upper Half Mean	Lower Half Mean		
0	1.01	0.86	1.08	3.6
10	0.97	0.83	1.08	4.5
100	1.02	0.88	1.05	4.1
500	1.00	0.84	0.98	4.0

The oil content of the embryos tended to increase stepwise with each increase in Thimet level, while the protein content was oppositely affected (table 5). The phosphorus content of embryos from the plants treated with 10 p.p.m. were 11.2% less than the controls, while those from the 100 and 500 p.p.m. treated plants were 1.5 and 11.0% greater, respectively.

Seed Viability.—Seeds from plants treated with Thimet had germination percentages comparable to the controls. Also, no differences in seedling vigor or morphology were evident.

Discussion.—Under the experimental conditions here-

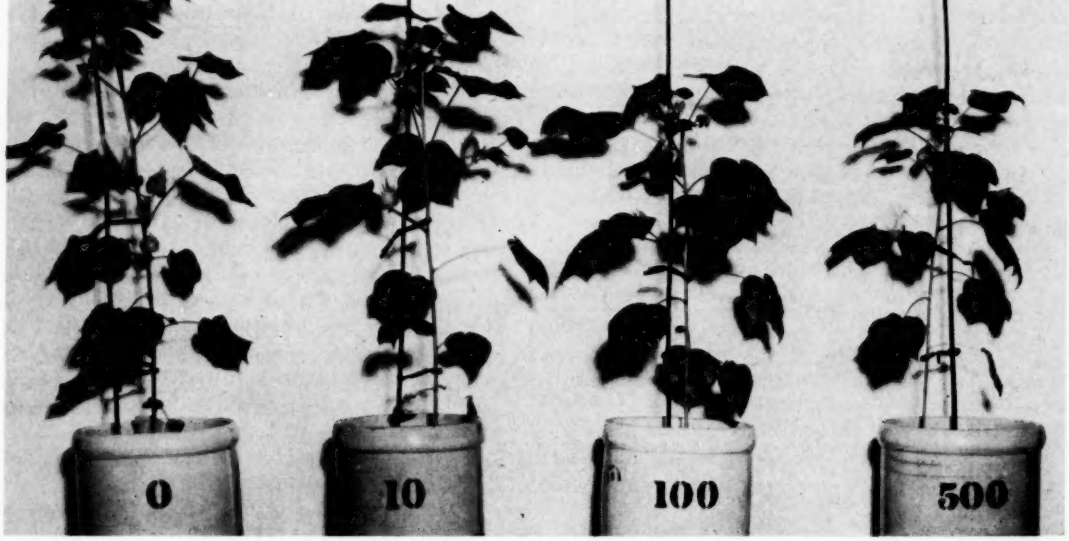

Fig. 2.—Cotton plants at 52 days on 0, 10, 100 and 500 p.p.m. of Thimet.

Table 4.—Influence of Thimet on dry weights, carbohydrate and nitrogen fractions (entire plants), and on the phosphorus fractions (leaves) of 20-day-old plants.[a]

Thimet Level in Substrate (P.P.M.)	Dry Weight per Plant (Gms.)	Carbohydrates[b]			Nitrogen[b]		Phosphorus[b]	
		Reducing Sugars	Sucrose	Starch	Soluble	Protein	Chloroform Soluble	Chloroform Insoluble
0	1.42	0.94	0.60	8.51	1.02	3.17	0.0035	0.1047
10	1.15	2.91	1.19	10.24	.90	2.72	.0076	.1363
100	1.00	3.86	1.82	10.57	.90	2.59	.0075	.1863
500	0.88	3.90	1.96	12.97	.85	2.55	.0124	.1678

[a] Results on a dry weight basis.
[b] Expressed on a percentage basis.

in reported, the initial wilting response of all plants treated with Thimet was an immediate effect and appeared to be caused by a reduction in water absorption, even though adequate moisture was available. One might conclude that respiration was inhibited, but unpublished data obtained by the author indicate that the respiratory rates of leaf discs from plants treated with Thimet were not significantly affected. Hanna (1956) reported that Thimet at rates of 1.5 and 3 pounds per hundred pounds of seeds caused reductions in emergence and induced wilting in seedlings under field conditions. Some of the seeds in the above mentioned field experiment which had completely failed to germinate were removed from the soil by the author and placed in moist petri dishes in the laboratory. Immediate germination resulted. Since adequate moisture enhanced germination in this case, it is conceivable that a slight moisture stress and a slight phytotoxic effect by the insecticide could possibly prevent germination or emergence and induce wilting. The exact nature of this phytotoxicity appears to be complex; consequently, studies are currently underway to determine the mode of action of this and other organo-phosphorus insecticides in the cotton plant.

It has been shown elsewhere that approximately 80% of all schradan absorbed through the root systems of the cotton plant accumulate in the leaves and necrosis was present at phytotoxic concentrations (Hacskaylo & Ergle 1955). If the degree of necrotic flecking found on leaves of plants treated with Thimet can be taken as a measure of Thimet accumulation, it would appear obvious that large quantities are translocated and deposited in those areas. Since most of the necrotic spots were found in the interveinal areas of the leaves, visual observations indicate that the toxic material was accumulated at the vein endings. Direct contact of the phytotoxic principle with the plasma membranes of the cells in the spongy layer would then be posssible. The sequence of events which lead to leaf necrosis follows a definite pattern. The first visual symptom is the water soaked appearance of cells which is followed by collapse of the cell walls and complete desiccaation of the protoplasm.

Since the number of seeds per boll was less in the two highest treatments (100 and 500 p.p.m.) than in the controls and 10 p.p.m. plants, a reduction in fertility may have resulted. It may appear that the insecticide had a stimulatory effect on seed development since the seed index increased with increasing levels of Thimet. It is most likely however, that the increase in seed weight was due to smaller bolls with fewer but larger seeds rather than due to a specific stimulatory effect of Thimet since the largest seeds were obtained from a phytotoxic concentration of the insecticide (100 p.p.m.).

Under the present experimental conditions, the plants treated with 10 p.p.m. of the insecticide did not show any marked deleterious effects on vegetative or fruiting properties as compared with the controls.

The reduction in soluble and protein nitrogen in the young treated plants was probably due, in part, to a reduction in nitrogen absorption by the root systems; however, the explanation awaits further experimental data. On the other hand, the chloroform-soluble and chloroform-insoluble P of the leaf increased. Originally, it was thought that a partial fractionation of the leaf phosphorus might provide a measure of Thimet accumulation by cotton because normally the chloroform-soluble (lipid) phosphorus of the leaf is consistently small, and the systemic with 11.9% P is readily soluble in this solvent. However, since increases were found in both fractions, it seemed unwise to attempt to differentiate between Thimet and other phosphorus constituents of the plant.

Since accumulation of reducing sugars, sucrose and starch has been shown to occur in plants treated with Thimet, it would appear that the enzyme systems controlling carbohydrate utilization are in some way affected. Yet unpublished data from this laboratory indicate that crude enzyme preparations of phosphorylase, phosphatase, and amylase from untreated cotton leaves are not inhibited by Thimet additions up to and including 3,000 p.p.m. Also, preliminary experiments with detached leaves from plants treated with Thimet show that their sugar reserves are depleted when placed in the dark for 24 hours. Although the rate of carbohydrate depletion was not measured, it was significant that the leaves were able to oxidize these reserves even though some were from plants treated with concentrations as high as 500 p.p.m. This suggests that these enzyme systems of treated

Table 5.—Influence of Thimet on the oil, protein and phosphorus content of cotton seed embryos.[a]

Thimet Level in Substrate (P.P.M.)	Oil	Protein (N×6.25)	Phosphorus
0	31.92	43.75	0.582
10	33.45	41.85	0.517
100	34.78	41.68	0.591
500	34.54	41.11	0.646

[a] Expressed on a percentage basis.

plants were retarded, but not completely inhibited. These results are in contrast with those obtained for an enzyme system such as cholinesterase which is drastically inhibited with as little as 10 p.p.m. of Thimet. Lord (1955) has presented some evidence which shows that a reduction in esterase activity occurs in some plant species when sprayed with Systox (demeton), Hanane (dimefox) and parathion. He has further stated that this enzyme inhibition may, or may not be associated with phytotoxicity. Thus, in plants, in contrast to animals, current evidence does not permit the conclusion that any one enzyme system is responsible for the phytotoxic response caused by organo-phosphorus insecticides.

SUMMARY.—Cotton plants were gown to maturity in the greenhouse in sand cultures containing 0, 10, 100 or 500 p.p.m. of Thimet. All plants receiving the insecticide initially wilted but recovered. Slight (10 p.p.m.) to severe (500 p.p.m.) necrotic flecking of the leaves occurred and was correlated with levels of Thimet in the substrate. Final dry weights were slightly greater for plants grown on the lowest level of Thimet (10 p.p.m.) but were successively less on the 100 and 500 p.p.m. concentrations as compared with the controls.

The yield of seed cotton was comparable for the plants grown on the 0, 10, and 100 p.p.m. levels of Thimet but the boll weights and number of seeds per boll decreased at the two highest rates as compared with the 0 and 10 p.p.m. series. Larger embryos were produced by plants receiving the insecticide.

Reducing sugars, sucrose and starch accumulated in young plants treated with Thimet and soluble and protein nitrogen decreased. Chloroform soluble and insoluble phosphorus in leaves of young plants tended to increase with increasing levels of Thimet.

Thimet tended to cause an increase in the oil content of embryos at the expense of protein formation.

Seeds harvested from plants in all treatment levels had a germination percentage of about 92%.

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Schizura ipomaeae Dbldy. Attacking Plums in California[1]

HAROLD F. MADSEN and STANLEY C. HOYT, *University of California, Berkeley*

In the late summer of 1953 a report was received of an infestation of unknown larvae in a plum orchard near Marysville, California. Upon investigation, it was discovered that the larvae were feeding upon foliage of Santa Rosa plums and a considerable acreage in the orchard was involved. Complete to partial defoliation of the trees had taken place, and the larvae were mostly near maturity. A tentative identification of the larvae was secured, and it was thought at that time to be the unicorn caterpillar, *Schizura unicornis* (J. E. Smith). The following season, when it was possible to secure adults as well as larvae, they were identified by E. L. Todd of the Entomology Research Branch, U. S. Dept. of Agriculture, as *Schizura ipomaeae* Dbldy.

As far as is known, this is the first record of *S. ipomaeae* attacking a commercial crop in California. Since the original description of the species by Doubeday in 1841, there has been little written except for descriptions in various monographs of the Lepidoptera. It has been recorded from the eastern states, and the only record of distribution from the west has been from Idaho by Manis (1954). There is also an unconfirmed report of its occurrence in Utah.

Peterson (1948) lists the host plants as oak, maple, birch, blackberry, and honey locust. Seitz (1924) lists in addition to the above hosts, elm, *Rubus*, *Vaccinum* and *Ceanothus* in the northern states, the *Ipomea coccinea* in the Gulf states. In Idaho it was recorded from prune, and in California the only known host is plum.

The mature larva is very distinctive, and exhibits protective coloration to a high degree (fig. 3). The head is purple, with four dark lines from the mandibles to the vertex. The meso- and metathorax are green, and the body brick red to brown in color. Mature larvae characteristically feed along the margins of the leaf and remain motionless when disturbed. The body color of the larva gives the impression of a leaf with brown or reddish edges, and the green thoracic area adds to the illusion. They are very difficult to distinguish from leaves which possess brown edges, and are easily overlooked. The larvae have a dorsal projection on the first abdominal segment which is bifurcate, each projection bearing a spine on the tip. *Schizura unicornis*, a closely related species has much the same larval coloration, but the dorsal projection is not bifurcate, and bears a single spine.

The adult has ashy gray forewings with streaks of brown, and the hindwings are dirty white. The moth, when at rest, holds the forewings in an inverted V-shape over the abdomen, and when resting on the trunk of a tree is almost impossible to see (fig. 4).

Over the past three seasons, observations have been made on its seasonal history in the field. There are many details yet to be worked out, but a fairly complete life history has been obtained. Attempts to rear the species in an insectary have been only partially successful, but indications are that it could be successfully reared under proper conditions.

The infestation thus far has been limited to plums in

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the one orchard near Marysville. About 400 acres are involved, and the larvae seem to prefer the Japanese varieties such as Santa Rosa, Duarte, and Kelsey. Only an occasional larva has been found on the blue plums such as President and Tragedy. In this orchard, plums are planted adjoining blocks of Bartlett pears, but no larvae have been found feeding on pears. Although there is considerable acreage of both plums and prunes in the Marysville area, the infestation has been limited to this single orchard.

SEASONAL HISTORY.—*Schizura ipomaeae* overwinters as a pre-pupa in the soil (fig. 1). When larvae reach maturity in September, they drop from the trees and enter the soil. The larvae lose their characteristic color, become grayish brown, and shorten in length. They construct cells in the soil, consisting of a hollowed-out area surrounded by a loosely constructed case of soil particles held together with silk, The soil in the infested orchard is very sandy, and it is not known if the larvae would be able to penetrate packed or heavy soils. The overwintering forms are usually found 1 to 2 inches beneath the surface, and they seem to prefer areas with a ground cover of grass. Occasionally they are located deeper in the soil, but they are rarely present below a 6-inch depth. They may be found in an area extending from the trunk of the tree to the drip line. When disturbed, the pre-pupa will become active and move about. If removed from their cells, and placed on the surface of the soil, they will again enter the ground and construct new cells. Since the orchard involved is mostly clean cultivated, late fall disking may disturb the pre-pupa, but it does not seem to reduce the overwintering population to a significant degree. This is probably because of their ability to reconstruct overwintering cells.

In April, the pre-pupa transforms to the pupal stage within the overwintering cell. Pupae are uniform dark brown in color and resemble the pupae of other soil-inhabiting Lepidoptera such as the cutworms.

Moths begin emergence in May, the earliest record being May 10. Little is known concerning the activity of the moths. They were occasionally found resting on the trunks of the trees, and when disturbed would fly rapidly to another tree, remaining close to the ground. Since no activity was observed during the daylight hours, it seems probable that mating and egg-laying take place in the late evening or at night.

Eggs are laid singly on the undersides of the leaves and usually only one egg per leaf is found. The eggs are round, transulcent, with a faint reticulation (fig. 2). The majority of the eggs are laid on the leaves of sucker shoots in the center of the trees, although they are also found on the leaves of the main branches and shoots. Shortly after hatching, the young larvae begin feeding on the lower leaf surface, in such a manner that the top epidermis remains intact. This superficial feeding on the lower surface forms small "windows" in the leaves. This type of feeding is very characteristic, and enables one to detect readily the presence of young larvae.

As the larva matures, it moves to the leaf margins and devours the entire leaf from the margin towards the midrib. The habit of feeding on the margin allows the larva to take advantage of the protective coloration previously described. The larvae consume the leaf surface with the exception of the midrib, and feed from the tips of the branches downward. In heavy infestations, the trees are characteristically defoliated from the tops downward. Observations in the laboratory indicate that the larvae pass through five instars. The first two instars feed on the undersides of the leaves, and the last three feed at the leaf margins.

FIG. 1.—Overwintering pre-pupae in soil.

When handled, the mature larvae exude a liquid from the thoracic region which has a very pungent odor resembling that of formic acid. This reaction probably serves to deter predators, and it has been noted that birds will not attack the larvae.

In late June, the larvae reach maturity and drop to the ground. Pupation takes place in the soil, and adults of the second generation emerge in mid-July. The first and

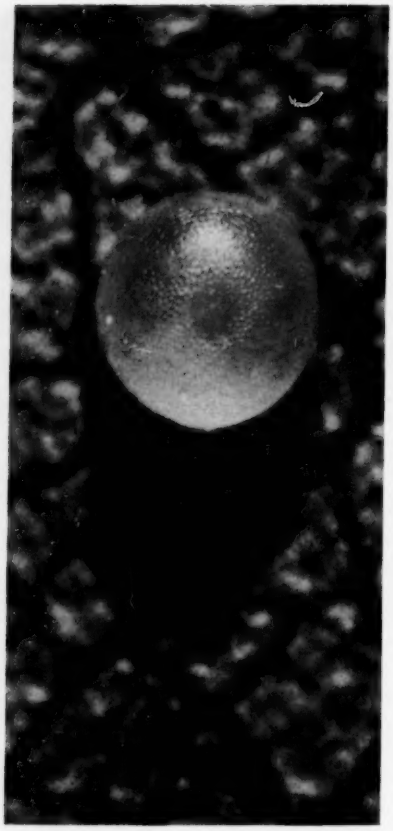

FIG. 2.—Egg of *Schizura ipomaeae*. Greatly enlarged.

second generations are somewhat overlapping, and mature larvae of the first generation are often found with young larvae of the second generation.

Eggs of the second generation are usually laid on the sucker shoots, but are more commonly found throughout the tree than are the first generation eggs. Larvae of the second generation reach maturity in late August and September, and drop to the soil to enter the overwintering stage.

The second generation provides the heaviest infestation, and is the one that causes commercial damage. The first generation larvae are often overlooked, as they tend to stay in the centers of the trees. The second generation comes after the plum harvest, and again may not be noticed unless leaf damage becomes serious. Larvae have not been observed to attack the fruit, as all of their feeding is restricted to foliage.

CONTROL.—This insect was easily controlled with a spray of DDT directed against the young larvae in late May or early June. The mature larvae, however, were difficult to kill, and even combinations of DDT and parathion did not appreciably reduce the populations. Since the young larvae were generally located in the center of the trees, and the mature larvae were so protectively colored, infestations were not normally noticed until excessive feeding had taken place. Once an infestation was known to exist, however, sprays could be timed according to the appearance of the "windows" cut in the leaves by the young larvae.

FIG. 3.—Mature larvae on leaf.

In the Marysville orchard, populations have continued to appear each season in spite of the spray program directed against them. Populations have been low since the original heavy infestation, but injurious numbers could possibly develop if chemical control were abandoned. There are undoubtedly a number of ecological factors which exert an influence upon the populations, as considerable fluctuation in population densities occurred in a small area of orchard which was left untreated in order to allow observations on the seasonal activity of the insects.

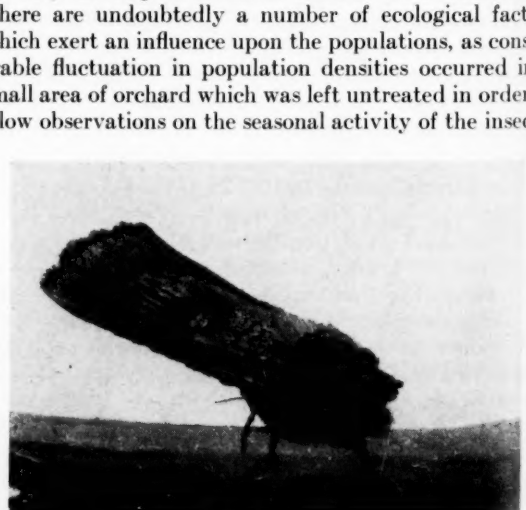

FIG. 4.—Adult of *Schizura ipomaeae* in natural resting position.

These factors, however, have not been ascertained. A considerable number of larvae have been collected for laboratory rearing attempts over the past three seasons, and not a single parasite has been reared from these collections. The relationship of parasites to this species therefore remains unknown.

During the winter of 1955–56, a severe flood occurred in the Marysville-Yuba City area. The orchard was washed out in some areas, while in others 2 to 3 feet of silt was deposited. In spite of this, the larvae appeared in the orchard the following spring in numbers exceeding that of the previous year. This indicates the ability of the species to endure rather severe winter conditions; but its ability to survive extreme cold cannot be determined, as temperatures in the infested area rarely drop below freezing in normal winters. Studies will be continued on *Schizura ipomaeae* in order to secure more detailed information on its biology and to ascertain the effects of ecological factors on population densities.

SUMMARY.—An infestation of larvae on plums in the Marysville district of California was reported in 1953, and the insect involved was determined as *Schizura ipomaeae* Dbldy. Considerable damage was done to the plums as a result of larval feeding and complete defoliation was observed on some trees.

Field observations over three seasons resulted in a fairly complete knowledge of the life history. The insect overwinters as a pre-pupa in the soil, and pupates in April. Moths emerge in May and lay eggs on the undersides of the leaves. Young larvae feed on the lower leaf surface, and older larvae feed on the leaf margins, eating to the midrib. A second generation appears in July and August, the larvae of this generation dropping to the soil in September to overwinter.

Control can be obtained by the use of DDT directed against the first generation larvae.

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The Pennsylvania Insect Light Trap[1]

S. W. FROST, *The Pennsylvania State University, University Park*

During the past 10 years, 50 different combinations of baffles, funnels, lamps and killing jars have been tried by the writer in an attempt to produce a more efficient insect light trap. Many of these developments have shown little or no advantage over traps previously used and were soon discarded. A few have shown promise and are worthy of special mention. One in particular, which has been designated the "Pennsylvania insect light trap," has proved outstanding and has been used by the writer for several years as a standard for comparison with other traps.

Two well-known traps that have been used quite generally should be mentioned. The New Jersey mosquito trap is still recognized as the most efficient for its intended purpose. Catches from this trap were compared with the Pennsylvania trap during 1956. The Minnesota trap is especially useful where bulb-type lamps are used. Reports on the efficiency of this trap were published by the writer (Frost, 1953, 1954).

DESCRIPTION OF TRAPS.—The Pennsylvania insect light trap (fig. 1) was constructed entirely of 20-gauge sheet aluminum, weighed 4 pounds and cost less than $15.00. It consisted of four baffles 20 inches long and 5½ inches wide, a 60°-funnel, 12 inches in diameter and a flat circular top 25 inches in diameter. The funnel narrowed to 2 inches at the lower end and was fitted with a threaded Mason jar top to accommodate the killing jar. It was equipped with a central 15-watt black-light fluorescent lamp and the proper ballast and starter.

It is well known that covers slightly reduce the catches of insects at traps. They are necessary to eliminate rain from the killing jar and are especially important when the catches must be sorted and identified. Special rain deflectors have been used by some designers but have not proved satisfactory. In surveying for *Phyllophaga* and similar beetles, the writer successfully operated light traps without tops using a quart jar half-full of kerosene as the killing bottle. *Phyllophaga* taken from such traps were soft and in excellent condition even after being shipped in plastic bags from a distant point.

Studies were made to improve previous types of traps, to simplify their construction and to increase the catches of the larger insects especially Noctuidae, Tortricidae, Crambidae and certain Coleoptera. Traps incorporating many new ideas have been built and tested. The construction and operation of a few of these are discussed. They are designated experimental traps[2] and are indicated in the tables by numbers as follows:

No. 1. The same construction as the Pennsylvania trap except the lamp and baffles were lowered 4 inches below the rim of the funnel. This was an attempt to make a more compact trap.

No. 2. Similar in construction to the Pennsylvania trap except two 8-watt fluorescent lamps were mounted in the center between the baffles which were shortened to 15 inches. This was another attempt to produce a more compact trap (fig. 2).

No. 3. An aluminum trap with four baffles, 15 inches in length and 6 inches in width intersecting at the center, a 12-inch 60°-funnel and a 25-inch circular top. This trap was equipped with four 8-watt black-light fluorescent

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[2] Many contributions to the design, construction and operation of these light traps were made by George H. Beatty during his connection with the project in 1955 and 1956.

lamps placed in the angles formed by the intersecting baffles (fig. 3).

No. 4. Similar in construction to trap No. 3 except the baffles were covered with dull-black paper.

No. 5. Similar in construction to traps Nos. 3 and 4 except the baffles were constructed entirely of $\frac{1}{8}$-inch plexiglass.

No. 6. An aluminum trap formed by two 90°-funnels each 15 inches in diameter. The upper funnel served as a baffle. A 32-watt black-light fluorescent circline lamp, in a horizontal position, was placed so that it was visible just above the upper edge of the lower funnel. The lower

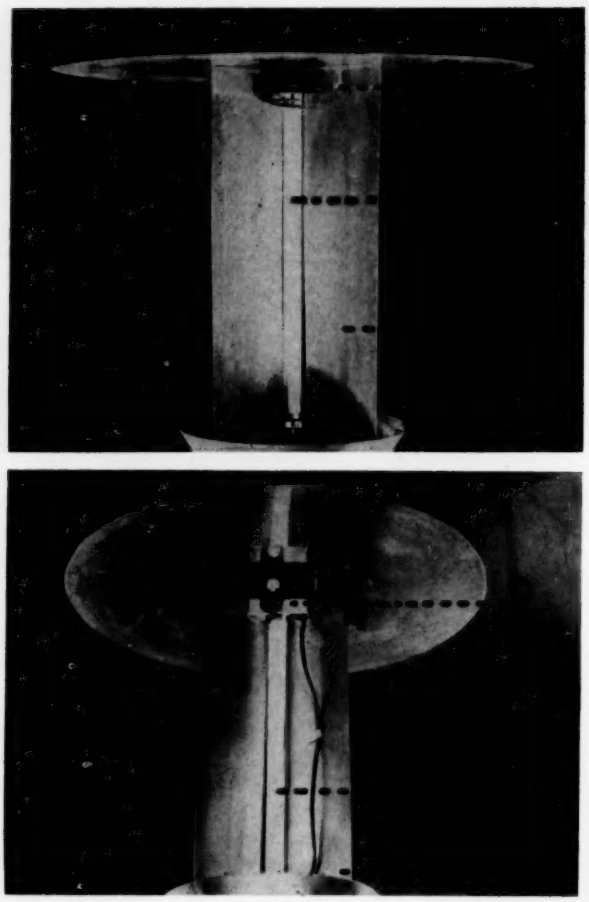

FIG. 1.—Two views of the Pennsylvania Trap showing A 15-watt fluorescent lamp, B baffles, C ballast and D starter.

funnel directed the insects into the killing jar at the bottom of the trap (fig. 4).

No. 7. Similar to the above in construction except the upper funnel was replaced by a plastic cylinder which served as a baffle and the circline lamp was placed well above the rim of the funnel (fig. 5). It is unfortunate that this experiment was conducted for only 2 nights during a period of low temperatures resulting in comparatively small catches. This is an interesting trap that was easy to build but more expensive than many. It was equipped with a large fluorescent lamp, not at present available on the market, and a large ballast.

No. 8. Similar to No. 7 except the cylinder was covered with dull-black paper.

FIG. 2.—Trap with two 8-watt fluorescent lamps placed between the intersecting baffles. (Expt. No. 2)

No. 9. A trap with the 32-watt black-light circline fluorescent lamp placed in a vertical position with a circular aluminum baffle filling the opening in the center of the lamp and a second baffle of the same size and shape at right angles to the first (fig. 6). The temperature was low during the 2 nights this experiment was conducted and the catches were correspondingly small. Little difference was observed between the catches from these two traps. The number of insects taken in the Pennsylvania trap was 468 and 550 were taken in the trap with the 32-watt circline lamp.

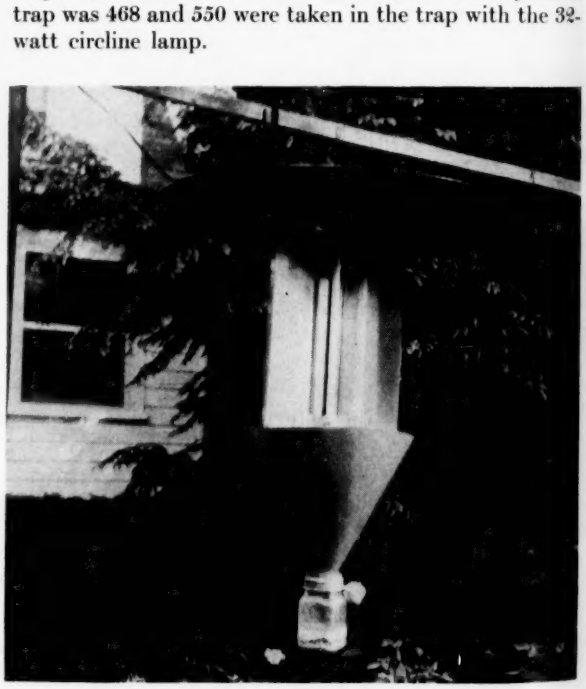

FIG. 3.—Trap with four 8-watt fluorescent lamps in the angles of the four intersecting baffles. (Expt. No. 3)

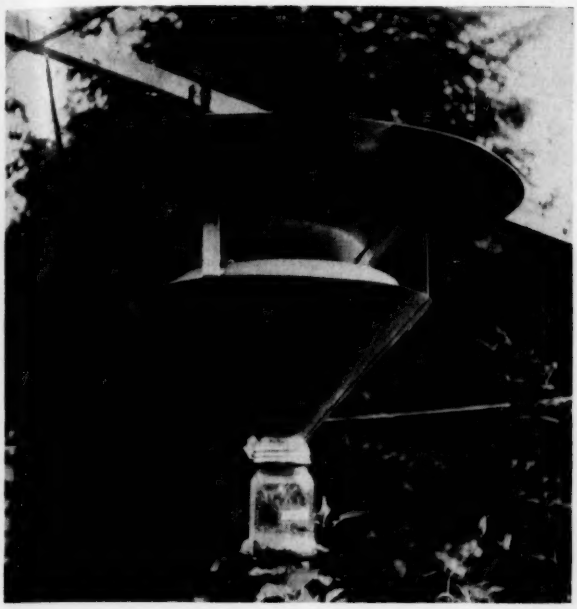

FIG. 4.—The two-funnel trap with 32-watt circline lamp in a horizontal position. (Expt. No. 6)

No. 10. Similar to trap No. 9, except a square baffle was added outside the fluorescent circline lamp (fig. 7).
No. 11. Similar to the Pennsylvania trap with an electric fan mounted above the baffles. This was not an ideal arrangement. The aluminum funnel formed a dead-air area at the bottom of the baffles. For the best results, a wire-screen funnel would seem desirable. Nevertheless, the catches were considerably increased in the 3-night experiment. With the fan, 4026 insects were taken, without the fan, 1971 insects were taken. The chief difference occurred in the midges of which 2996 were taken with the fan and 1212 without the fan.
No. 12. Trap identical to No. 2 except the baffles were painted dull-black. In this experiment black baffles did not increase the catches. The number of insects captured in the trap with unpainted baffles was 2274 and 918 were taken in the trap with black-painted baffles. The experiment was conducted for only 2 nights and the figures may not be significant.

Black-light fluorescent lamps[3] were used in all traps except the New Jersey trap which was equipped with a 100-watt inside-frosted tungsten lamp. Three types of fluorescent lamps were used (1) a 15-watt lamp, 18 inches in length (2) an 8-watt lamp, 12 inches in length and (3) a 32-watt circline lamp, 12 inches in diameter. The latter was provided by the General Electric Company for special tests.

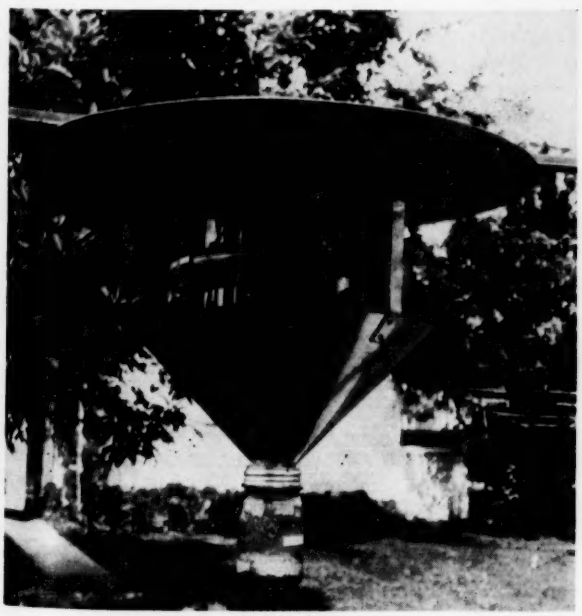

FIG. 5.—Trap with a plastic cylinder as a baffle and a 32-watt circline lamp in a horizontal position. (Expt. No. 7)

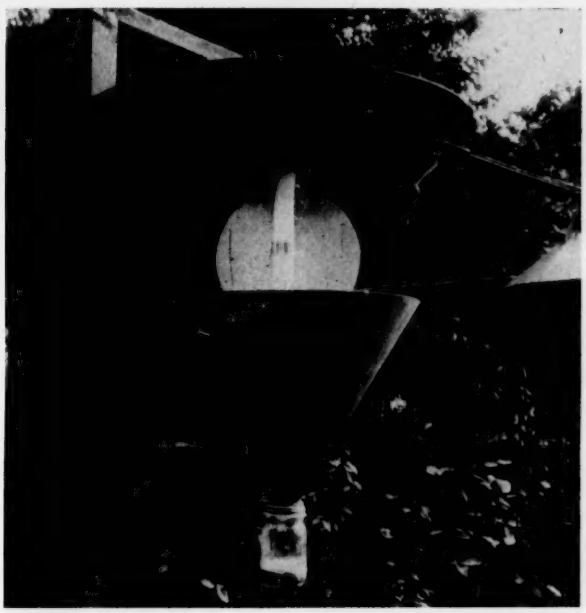

FIG. 6.—Trap with 32-watt circline lamp in vertical position and with two round baffles. (Expt. No. 9)

OPERATION OF TRAPS.—All traps were operated in a small area in State College, Pa. They were hung on one or two standards and were usually operated in pairs. The distance between individual traps was never more than 2 feet. If more than two traps were tested at the same time, the second pair was hung on another standard about 15 feet distant and somewhat shielded from the first by foliage. When the operation of traps exceeded 7 days, their positions were interchanged at least once a week so that none benefited by its position. All lights were simultaneously controlled by a time switch. The period of operation averaged about 8 hours a night varying with the intensity of the daylight at various periods of the summer. The operation period was not significant because all comparative tests were conducted for the same number of hours on identical nights. All traps were compared with the Pennsylvania insect light trap.

[3] These General Electric lamps contain a special phosphor whose radiation peaks around 3500 Angstroms in the near ultraviolet region.

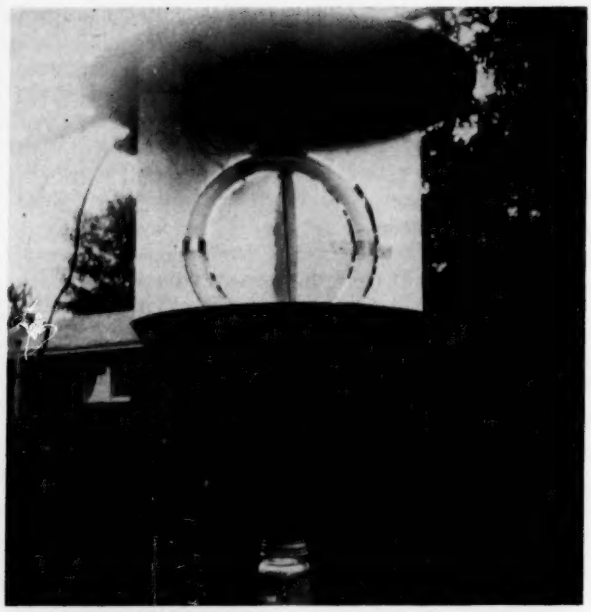

FIG. 7.—Trap with 32-watt circline lamp in vertical position and with extra baffles outside the lamp. (Expt. No. 10)

The insects collected every morning from each trap were immediately sorted and identified to orders, families, and (in the case of some economic forms) to species. Many of the species that were identified, especially the *Phyllophaga* and Noctuidae, are not indicated in the tables.

DISCUSSION.—Many complex lighting problems, other than quality and intensity are only partly solved. The effects of light intensity will be reported in a separate paper. Tests with various types of baffles have been conducted to determine the effect of reflections. Aluminum, plastic and plexiglass cause reflections which apparently reduce the catches of insects. The Pennsylvania trap is constructed with the lamp in the center so that there is little or no reflection from the baffles. The same is true of experimental trap No. 2 with two 8-watt lamps mounted in the opening between the four intersecting baffles. In the case of experimental trap No. 3, in which a lamp is placed in each of the four angles formed by the baffles, the transmitted light is reduced and that reflected is increased. In 1955, green fluorescent paint[4] on the baffles of one trap and a proprietary trap with green fluorescent baffles gave consistently smaller catches.

Black baffles were tried on many of the traps to reduce reflections. In general the results were satisfactory and especially so in the case of experimental trap No. 4.

Another factor worth consideration is the absorption of ultraviolet radiation by aluminum. At 3100 Ångstroms, which include part of the output of the black-light fluorescent lamps, from 60 to 70% of the ultraviolet radiation is absorbed. In the Pennsylvania trap, as noted previously, the light does not shine on the baffles and there is little or no reflection or absorption.

It has been surmised that heat produced by high wattage tungsten lamps and the BH_4 lamps used during previous years, may add to the efficiency of the traps. One test was conducted for a short period during September when the night temperature averaged about 50° F. A Pennsylvania trap equipped with a small heater[5] placed in the funnel below the baffles was compared with a Pennsylvania trap without a heater. No significant difference was noted.

Results of counts of insects taken in various traps during 1956 are presented in table 1. Experimental traps 2 and 12 (results not included), equipped with two 8-watt fluorescent lamps, resulted in significantly lower catches. As the cost of construction of these traps was greater, they do not seem practical. Experimental traps 3, 4, and 5, all with 8-watt fluorescent lamps in the four angles formed by the intersecting baffles, resulted in no improvement in catches. They also were more complicated and expensive to build and are therefore not considered practical. Experimental trap 6, consisting of two funnels, caught fewer insects than the Pennsylvania trap and was soon discarded. Experimental traps 7 and 8, with cylindrical baffles, did not seem promising and were likewise soon discarded. The catches from experimental traps 9 (results not given) and 10, equipped with 32-watt circline fluorescent lamps, compared favorably with those of the Pennsylvania trap but it is questionable whether these traps because of higher construction costs will prove practical. Experimental trap 11 (results not included), with an electric fan above the baffles, increased the catch of midges but otherwise showed no improvement.

Some outstanding differences in the response of insects to white and ultraviolet light may be cited. A Pennsylvania trap with a 15-watt black-light fluorescent lamp was compared with a New Jersey trap equipped with a 100-watt inside-frosted tungsten lamp. The two traps were operated on the same standard within 12 inches of each other. Mosquitoes and Tipulidae were caught in much larger numbers at the white light. It might be expected that these small light-bodied insects would be sucked in by the fan of the New Jersey trap. However, aphids, which are smaller were caught in large numbers at the ultraviolet lamp. The larger insects, Noctuidae, Crambidae, Macrotrichoptera and Formicidae were more strongly attracted to the ultraviolet lamp.

SUMMARY.—For insects other than mosquitoes and Tipulidae, the Pennsylvania light trap was superior to any of the other traps.

Lowering the lamp and the baffles into the funnel of the Pennsylvania trap reduced the catches only slightly. This resulted in a more compact trap and one which was more rainproof than the former model. In the long run this modification may be an improvement.

Black baffles reduced reflections and increased the catches. In some cases black paper was used to which the insect could cling more readily and may account, in part, for the poorer results. Black painted baffles generally increased the catches.

Reflections from the baffles reduced the catches. This was particularly true of the plastic, plexiglass and bright aluminum baffles. When the lamp was placed in the center of the trap between baffles, there was little or no reflection.

Although the traps incorporating two 8-watt and the four 8-watt lamps were very compact, these traps did not compare favorably with the Pennsylvania trap. Traps

[4] 2105 signal green, Prang A. C. Co.
[5] A Snelly-Fisher Hotspotting unit.

Table 1.—Insects taken at light traps, State College, Pa., 1956.

Classification	Sept 6–14 (9 Days)		July 24–Aug. 4 (12 Days)	June 1–July 11 (30 Days)		June 29–July 1 (5 Days)		July 12–23 (11 Days)		Sept. 5–11 (6 Days)		Sept. 12–13 (2 Days)		Sept. 14–16 (3 Days)		Sept. 23–Oct. 5 (9 Days)		Sept. 12–27 (16 Days)	
	Pa. 1–15w	Exp. 1 1–15w	Pa. 1–15w	Exp. 2 2–8w	Exp. 3 4–8w	Pa. 1–15w	Exp. 4 4–8w	Pa. 4–8w	Exp. 5 4–8w	Pa. 1–15w	Exp. 6 1–32w	Pa. 1–15w	Exp. 7 1–32w	Pa. 1–15w	Exp. 8 1–32w	Pa. 1–15w	Exp. 10 1–32w	Pa. 1–15w	N.J. 100wt
Culicidae	37	50	27	13	14	4	0	0	1	4	33	11	7	3	29	8	130	95	114	606
Simuliidae	0	1	4	2	15	11	1	2	3	2	0	1	0	2	0	0	5	4	6	0
Tipulidae	39	38	61	60	132	164	5	7	46	44	34	19	14	15	31	17	76	55	143	774
Midges	2,206	2,153	9,798	7,359	18,691	16,354	1,731	1,658	5,079	5,739	1,842	653	206	57	1,036	275	1,679	1,817	3,640	2,350
Anthomyiidae	4	6	349	250	1,048	1,288	170	69	512	328	2	5	2	3	1	0	2	1	5	0
Drosophilidae	55	55	124	104	558	626	159	158	190	264	51	15	9	14	11	11	18	12	50	110
Lonchopteridae	72	69	49	31	186	255	29	72	54	20	58	39	5	4	24	3	6	7	38	8
Boboridae	229	227	964	769	1,119	1,296	728	836	643	738	228	137	2	4	54	58	29	33	85	8
Empididae	1	2	5	0	1,077	1,690	93	87	11	18	1	2	0	1	0	0	0	0	0	0
Misc. Diptera	107	170	348	373	502	664	69	107	516	330	63	101	36	48	73	40	387	263	634	392
Aphididae	69	58	107	116	2,238	2,340	189	343	97	102	69	50	3	3	28	14	258	241	300	35
Cicadellidae	134	294	1,117	582	2,330	1,284	903	593	313	221	171	206	6	4	64	42	79	76	157	123
Misc. Homoptera	41	51	22	25	47	41	31	23	5	9	20	17	1	0	27	15	16	5	47	8
Corixidae	5	0	76	62	161	186	23	24	28	38	4	1	0	0	5	0	12	11	17	0
Miridae	212	220	114	97	578	586	474	329	184	146	157	91	17	12	93	37	41	47	157	28
Misc. Hemiptera	11	8	153	142	82	56	108	55	136	154	11	3	0	0	0	2	1	0	1	0
Carabidae	32	20	303	233	983	813	128	114	213	128	12	7	1	0	15	6	1	2	19	1
Staphylinidae	97	77	205	143	566	550	150	276	91	87	114	43	0	0	22	9	1	2	24	0
Curculionidae	14	7	45	22	43	18	9	9	11	10	11	8	0	0	7	10	2	1	9	0
Elateridae	1	2	21	25	67	45	44	12	37	15	0	0	0	0	0	0	0	0	0	0
Bostrichidae	1	0	4	14	101	77	16	8	6	3	1	0	0	0	0	0	0	0	0	0
Scarabaeidae	0	0	6	14	63	114	5	11	4	4	0	0	0	0	0	0	1	1	1	0
Phallophaga	0	0	1	0	61	33	6	3	0	1	0	0	0	0	0	0	0	0	0	0
Aphodius	20	21	17	12	124	174	39	41	11	6	25	13	0	0	1	1	0	0	1	0
G. xanthomelaena	34	29	38	38	349	229	137	152	2	3	13	20	0	0	23	2	0	0	23	0
Misc. Coleoptera	425	288	1,027	741	1,967	1,485	543	412	546	484	440	218	15	3	53	28	16	13	120	8
Crambidae	145	151	266	198	719	688	153	168	194	127	139	140	31	12	42	26	16	24	104	50
Tortricidae	47	49	66	43	377	392	101	151	160	145	55	44	7	10	8	9	8	5	27	18
Carpocapsa pomonella	3	4	23	23	112	94	21	21	10	18	12	3	1	0	0	0	0	1	1	0
Noctuidae	538	500	448	305	1,848	1,585	292	293	509	391	512	286	109	70	177	81	413	467	861	192
Macro-Lepidoptera	123	122	454	320	941	869	192	278	411	255	91	71	38	32	42	27	55	81	161	55
Micro-Lepidoptera	52	109	427	314	1,417	1,552	275	316	322	368	85	50	4	6	12	11	15	8	35	37
Formicidae	781	444	144	127	43	43	11	14	33	47	289	204	0	0	760	497	2	5	763	9
Misc. Hymenoptera	53	77	238	210	387	412	86	135	189	216	47	57	4	3	25	30	49	50	86	53
Ephemerida	3	1	4	0	49	51	19	55	5	0	3	2	0	0	0	0	0	0	0	5
Neuroptera	12	10	16	9	13	15	8	15	12	6	13	7	2	0	0	0	3	2	7	6
Macro-Trichoptera	77	92	183	144	1,270	1,129	229	233	177	179	85	74	17	13	44	27	38	50	114	3
Micro-Trichoptera	8	10	32	20	324	315	162	179	70	78	8	4	0	0	4	1	1	1	6	4
Misc. insects	56	53	347	167	56	41	14	20	40	28	51	26	3	5	11	5	10	12	28	13
Total	5,734	5,518	17,633	13,107	40,598	37,769	7,353	7,279	10,869	10,756	4,751	2,628	543	324	2,720	1,292	3,370	3,391	7,604	4,896

with 32-watt circline lamps require larger ballast. The greater cost of these traps should be considered.

The New Jersey trap cannot be favorably compared with the Pennsylvania trap because the former is designed specifically to trap mosquitoes.

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Insecticides in Transplant Water for Prevention of Tobacco Flea Beetle Emergence[1]

C. B. DOMINICK, *Virginia Agricultural Experiment Station, Tobacco Research Station, Chatham*

During years when the overwintered adults of the tobacco flea beetle, *Epitrix hirtipennis* (Melsh.), are very numerous, severe injury may occur to newly set tobacco plants. This injury consists of foliage damage by the adults and root and stem injury from the larvae. The result of this damage may be incomplete stands and stunted plants which are very slow in starting growth. Damage of this type is very important in Southside Virginia, especially on the heavier soil types.

The use of insecticides such as aldrin, heptachlor, chlordane, or lindane in transplant water is a recommended practice for the control of the tobacco wireworm, *Conoderus vespertinus* (F.). Allen *et al.* (1954) have reported excellent results after several years of experimentation with insecticides applied in this way, and other investigators have also reported favorable wireworm control from this procedure (Begg 1952, Jewett 1954).

The application of insecticides in transplant water for the control of other insects attacking newly set tobacco has received only limited attention. Townes (1951) found that demeton applied in transplant water gave excellent control of the green peach aphid, *Myzus persicae* (Sulz.). Dominick (1954) has reported control of the tobacco flea beetle on newly set tobacco plants with demeton applied by this method.

This paper presents the results of experiments which were conducted during the seasons of 1954 to 1956, inclusive, to obtain further information on the efficiency of insecticides applied in transplant water in reducing the infestation of tobacco flea beetles on newly set plants.

PROCEDURE.—A randomized block design was employed and each treatment was replicated five times. Each plot consisted of three plants which were set during the normal period for setting tobacco. These were planted with a hand tobacco setter, and 100 ml. of water containing the insecticide was applied in the hole around each plant. The same amount of untreated water was applied to each plant in the checks. No foliage insecticides were used.

The natural population of flea beetles was allowed to feed on all plants for a period of 21 days. At the end of this period the beetles were removed from the plant in each plot which showed the most severe foliage feeding. These selected plants were individually covered with cages consisting of metal frames covered with a good grade of cheesecloth and designed so that observation and removal of beetles could be made from the top. The plants were examined daily for a period of 3 days after being caged and any stray overwintered adults removed.

Table 1.—Tobacco flea beetle emergence from soil around tobacco plants treated with insecticides in transplant water. Chatham, Va. 1954–1956.

INSECTICIDE	OUNCES PER 50 GALLONS OF WATER[a]	TOBACCO FLEA BEETLE	
		Average Number Emerged per Plant	Per Cent Reduction Over Check
	1954		
Heptachlor 25% WP	6	0.5	98
Lindane 5% EC	2	2.4	90
Chlordane 50% WP	8	4.4	82
Demeton EC[b]	2	14.0	43
Check (water)	—	24.5	—
	1955		
Aldrin 25% WP	4	0.4	98
Lindane 5% EC	2	1.8	93
Chlordane 50% WP	8	2.2	91
Lindane 25% WP	0.25	2.2	91
Heptachlor 25% WP	6	2.6	90
Dieldrin 50% WP	2	3.8	85
Demeton EC[b]	2	8.4	67
Check (water)	—	25.4	—
	1956		
Heptachlor EC[c]	4	2.0	94
Chlordane 75% EC	3	2.4	93
Aldrin 25% WP	4	2.8	92
Aldrin EC[d]	4	4.0	88
Heptachlor 25% WP	4	7.8	77
Chlordane 50% WP	4	11.8	65
Check (water)	—	34.0	—

[a] Formulation; wettable powder (WP) by weight, emulsifiable concentrate (EC) in fluid ounces.
[b] Two pounds of actual Systox per gallon.
[c] Two pounds of actual heptachlor per gallon.
[d] Two pounds of actual aldrin per gallon.

Examinations were then made at 2-day intervals during a period of 6 weeks for the removal of newly emerged adults. The foliage on the caged plants was kept pruned so that the beetles could more readily be observed and collected.

RESULTS AND DISCUSSION.—The materials used in the treatments and the results of the experiments are summarized in table 1. In the preliminary experiment in 1954 excellent control was obtained with heptachlor, good control with lindane, and fair control with chlordane. Demeton gave poor control.

In the 1955 experiment aldrin, dieldrin, and lindane in wettable powder form were added to the materials tested. At the dosages employed, the following materials gave excellent to fair control: aldrin w.p.; lindane e.c.;

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chlordane, w.p.; lindane, w.p.; heptachlor, w.p.; and dieldrin w.p. Again, demeton gave poor control.

The purpose of the 1956 experiment was to compare wettable powder and emulsion formulations of heptachlor, aldrin, and chlordane. Good control was obtained with heptachlor, e.c., chlordane, e.c., and aldrin, w.p.; whereas only fair control was obtained with aldrin, e.c., and moderate to poor control with heptachlor, w.p., and chlordane, w.p.

The data from these experiments indicate that where hand transplanters are used, the flea beetle control obtained by the use of insecticides in the transplant water would justify treotment by this method, even if wireworms are not a serious problem. The reduction obtained in even a light infestation should serve to reduce the number of flea beetles from which later generations may develop. This treatment should not, however, be considered as a substitute for the foliage application of an insecticide at setting time.

SUMMARY.—Experiments were conducted during 1954 to 1956 in which the soil around newly set tobacco plants was treated with several chlorinated hydrocarbon insecticides applied in transplant water in a hand transplanter. The results showed a high reduction in the number of tobacco flea beetles (*Epitrix hirtipennis* (Melsh.)) which emerged from the soil around the treated plants. Aldrin, heptachlor, lindane or chlordane, at the dosages employed, gave excellent to fair control when used as either a wettable powder or an emulsion formulation. Demeton was less effective than the chlorinated hydrocarbon insecticides.

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Influence of Season and Weather on Citrus Red Mite Populations on Lemons in Southern California[1]

L. R. JEPPSON, C. A. FLESCHNER, M. J. JESSER, and J. O. COMPLIN,[2] *University of California Citrus Experiment Station, Riverside*

The distribution and seasonal trends of populations of the citrus red mite, *Metatetranychus citri* (McG.), in California have been reported by various workers since 1902. Woodworth (1902) and Quayle (1912) indicated this mite was almost entirely a coastal pest in California, primarily during the spring months (April through June). In 1936 Boyce stated that this mite is limited in distribution by climatic conditions, occurring in the coastal and subcoastal areas where humidity is generally higher than in the interior valleys and that peak infestations normally occur in the spring (April through June) and frequently in fall (October to December) but may occur during exceptionally warm winters or unusually cool summers, whereas, in the San Diego section it commonly occurs throughout the year. The citrus red mite has become a serious pest in the valleys of southern California between the San Bernardino and coastal mountain ranges (Riverside and San Bernardino counties) and in the San Joaquin Valley since 1936.

DeBach *et al.* (1950) studied seasonal trends in citrus red mite populations on Valencia oranges in groves near Santa Ana (Orange County), and found that although populations reach a peak in May and June and again in October and November, relatively high infestations may occur throughout the winter. They showed a correlation between fluctuations in red mite populations and predator populations.

Henderson & Holloway (1942) observed that mite population peaks occurred during plant growth cycles. They showed that significantly greater numbers of eggs were deposited on young and medium-aged leaves than on older leaves. They also demonstrated that leaf injury caused by the feeding of the mites resulted in a high reduction in egg production and a slight increase in mite mortality. They pointed out that populations of citrus red mites often decrease rapidly when climatic conditions are apparently favorable and natural enemies are scarce. They attributed these rapid population declines to the leaf injury resulting from excessive mite feeding. Jeppson *et al.* (1953) found that citrus red mite populations on Valencia oranges increased most rapidly during the spring and fall growth cycles.

Woodworth (1902), Quayle (1912), and Boyce (1936) did not specify the variety of citrus upon which their observations were made. The studies of Henderson & Holloway (1942) and of DeBach *et al.* (1950) were all on Valencia oranges. There has thus been no known critical study of the fluctuations of mite populations on lemons.

Although the major cycles of new growth of lemon trees occur during the spring and fall, new growth may occur any time of the year. The extent of the growth during these cycles and even the time when they occur may vary from one locality to another, from one year to another, or from grove to grove within a locality, depending on such cultural practices as irrigation and pruning. Generally, however, there is a major growth cycle in

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April and May, which reaches maturity in June. There is normally a considerable amount of new growth during July and August, followed by another major cycle in September and October, which reaches maturity during November. December to March is usually a period of minimum growth in most lemon districts.

In laboratory studies Pendergast (1938) found that citrus red mite eggs failed to hatch when relative humidities were below 20% or when temperatures were raised to 100° F. Mite larvae failed to reach maturity when relative humidities were below 40%. In the field he observed that during conditions of high temperature and low humidity the greatest mortality was in the egg and larval stages.

Field observations (Boyce 1936, and others) indicate that the effects of such low humidities and high temperatures have an important adverse influence on mite populations.

This paper deals with the general citrus red mite population cycles resulting from the yearly seasonal climatic changes, and the effects of temperature and humidity in modifying the magnitude and duration of these population cycles.

MATERIALS AND METHODS.—Lemon orchards selected

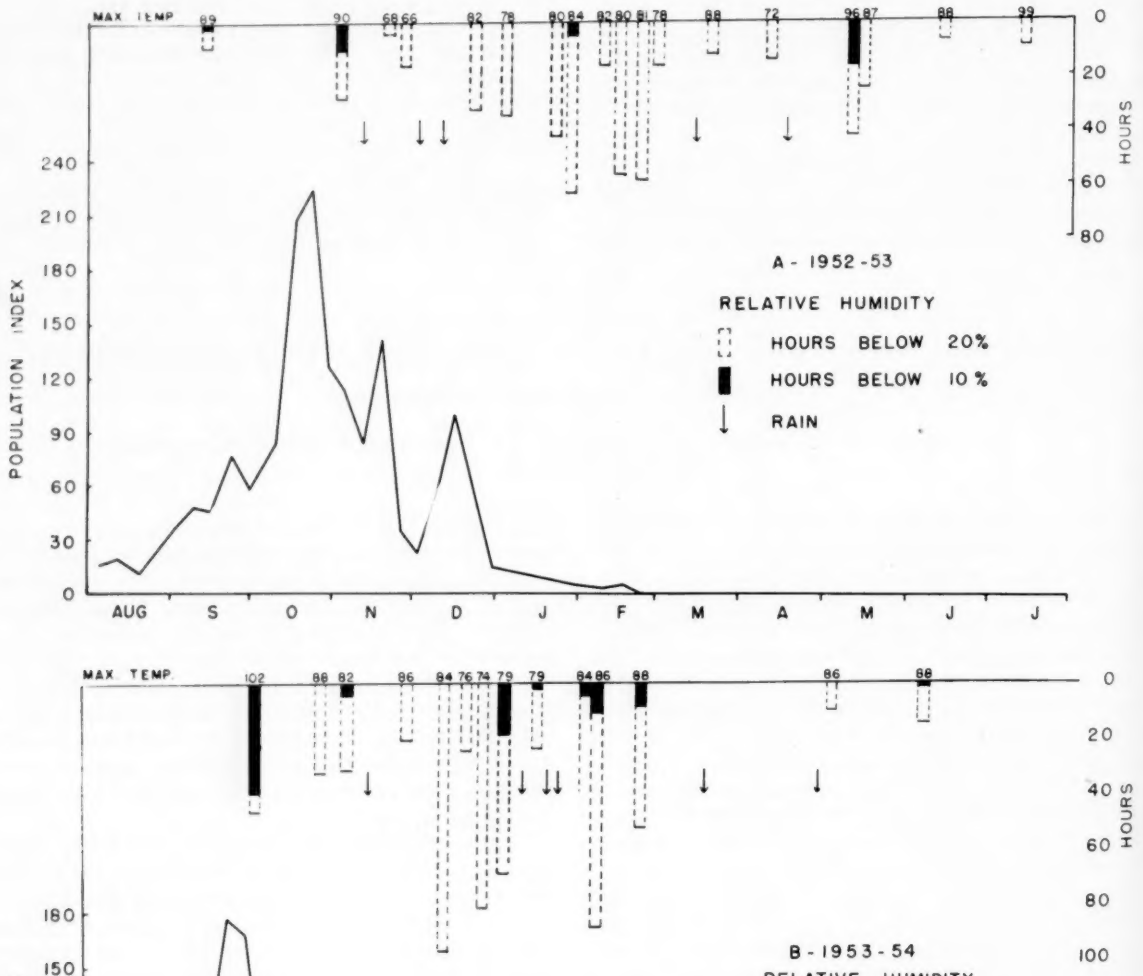

MAX. TEMP.
89 90 68 66 82 78 80 84 82 80 81 78 88 72 96 87 88 99
0 20 40 60 80
HOURS
240 210 180 150 120 90 60 30 0
POPULATION INDEX
A - 1952-53
RELATIVE HUMIDITY
HOURS BELOW 20%
HOURS BELOW 10 %
RAIN
AUG S O N D J F M A M J J
MAX. TEMP.
102 88 82 86 84 76 74 79 79 84 86 88 86 88
0 20 40 60 80 100
HOURS
180 150 120 90 60 30 0
POPULATION INDEX
B - 1953-54
RELATIVE HUMIDITY
HOURS BELOW 20 %
HOURS BELOW 10 %
RAIN
AUG S O N D J F M A M J J

FIG. 1. A and B—(*Continued on facing page*)

FIG. 1—(*continued*)

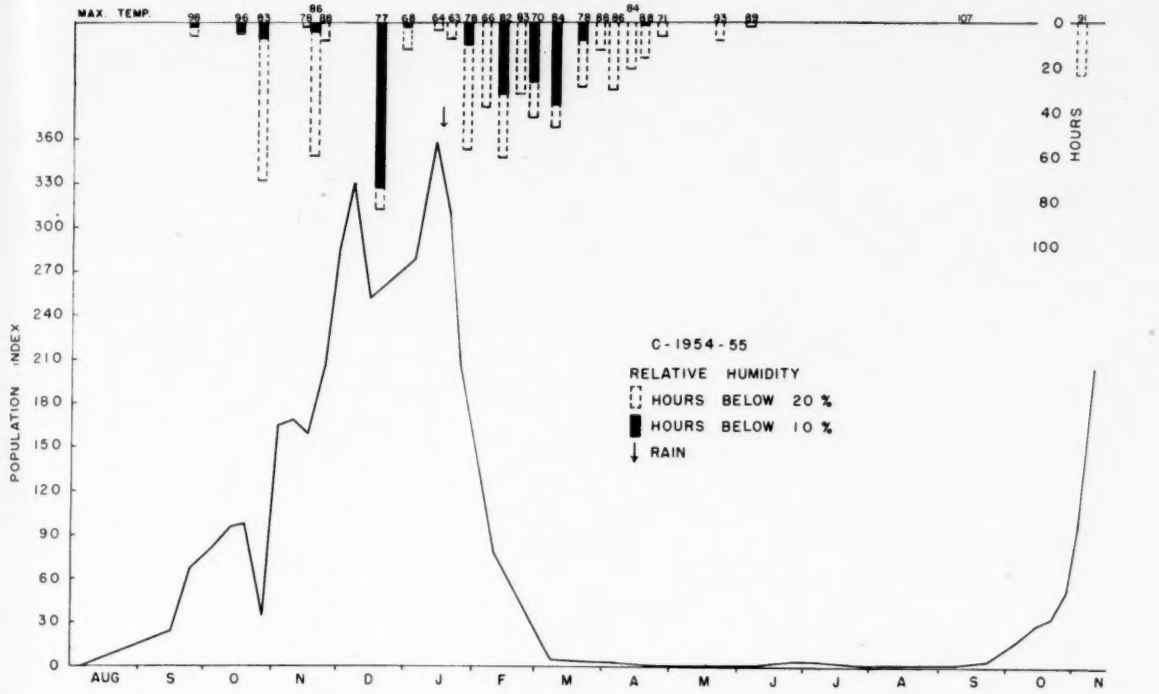


FIG. 1. A, B, and C.—Citrus red mite population trends on lemon trees in untreated plots in the Los Posas orchard (Ventura Co.) from August, 1952, to November, 1955. Bar graphs show periods of low humidity and maximum temperatures occurring during these periods.

for this study were the Lambert section of the Irvine orchard at Tustin, Orange County; the Los Posas orchard at Somis, the Utt Development orchard near Oxnard, and a Hardison orchard near Fillmore, all in Ventura County.

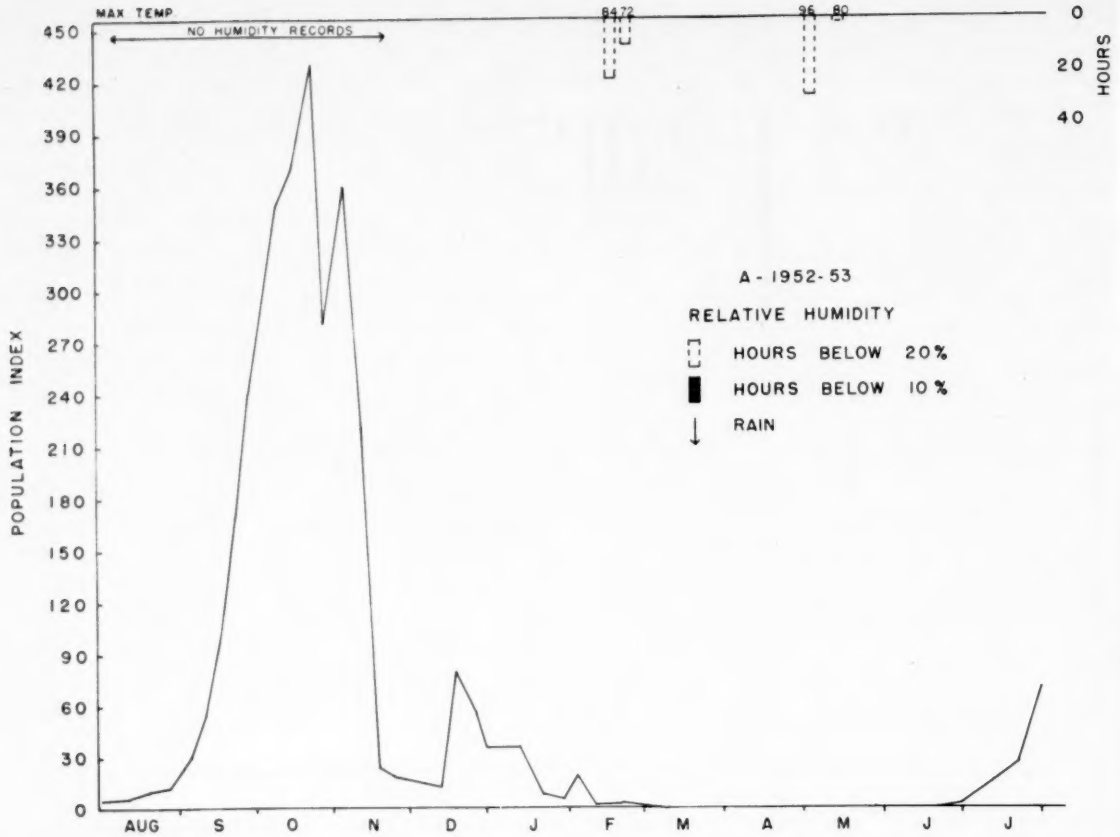
The Lambert orchard consisted of 5-year-old Rosenberger Lisbon lemon trees on sweet orange rootstock. Before the experiment was initiated the trees had been sprayed twice a year with California grade light-medium petroleum oil. The last treatment prior to the initiation of the experiment was on April 17, 1952. The Los Posas orchard consisted of 4-year-old Eureka lemon trees on sweet orange rootstock. The last treatment prior to the experimental studies was a similar type oil spray applied on February 23, 1952, and the experiments were initiated in August, 1952. The Utt Development orchard consisted of 17-year-old Eureka lemon trees on sweet orange rootstock, last treated with petroleum oil on April 14, 1949. The Hardison orchard had Lisbon lemon buds on sweet orange rootstock, and the last spray prior to these studies was with petroleum oil in August, 1949.

The citrus red mite population on each tree was evaluated at weekly intervals by counting the number of adult mites on the four full-sized leaves nearest the tip of the sample twigs. Two twigs were sampled from each quadrant of the tree. Results are recorded as average number of mites on the resulting 32-leaf sample per tree. In order to keep the intervals between samples comparable, evaluations were made on the same day of the week in each grove.

A hygrothermograph was maintained near the center of each grove for temperature and humidity records. Such records were made throughout the course of the experiment in the Los Posas orchard and, beginning November 8, 1952, in the Lambert orchard.

RESULTS.—Fluctuations in adult mite populations from August, 1952, to November, 1955, on trees in untreated plots in the Los Posas orchard are shown in figure 1, A–C. Mite populations in untreated plots in the Lambert orchard from August, 1952, to August, 1955, are shown in figure 2, A–C. Low relative humidities for the week prior to each mite evaluation date are indicated by bar graphs at the top of each figure. The shaded bars represent the number of hours during the week that the relative humidity was below 10%; unshaded portions indicate the hours that relative humidity was below 20%. The maximum temperature during the week is recorded at the top of the bar. Similar population studies were made during 1950, 1951, 1952, and 1955 on the Utt Development orchard near Oxnard (fig. 3), and during 1950 on the Hardison orchard near Fillmore (fig. 4).

DISCUSSION.—The seasonal population trends resulting from these studies indicate that unless adversely affected by periods of high temperatures and low humidities September and October and November were very favorable months for the increase of mite populations at the Los Posas, Lambert, and Hardison orchards (semi-coastal districts) and June through September were most favorable at the Utt Development orchard which was located near the coast (figs. 1–4). Each year in all four orchards, mite populations were either low or on the



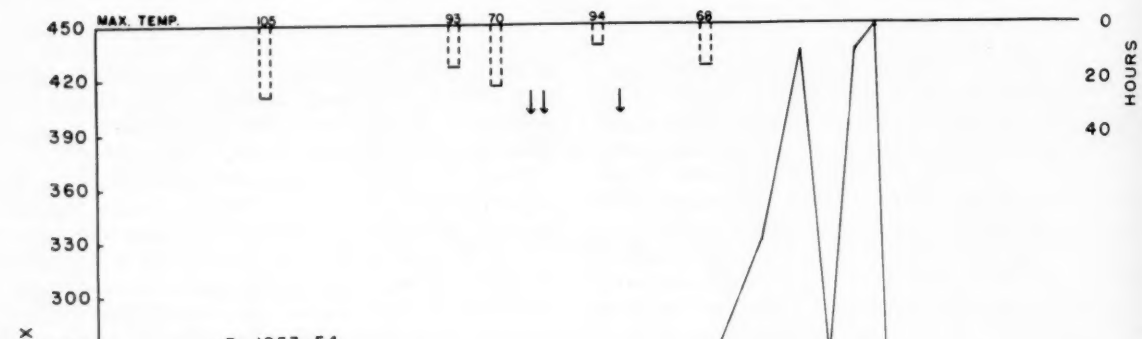


FIG. 2. A and B.—*(Continued on facing page)*

FIG. 2—(*continued*)

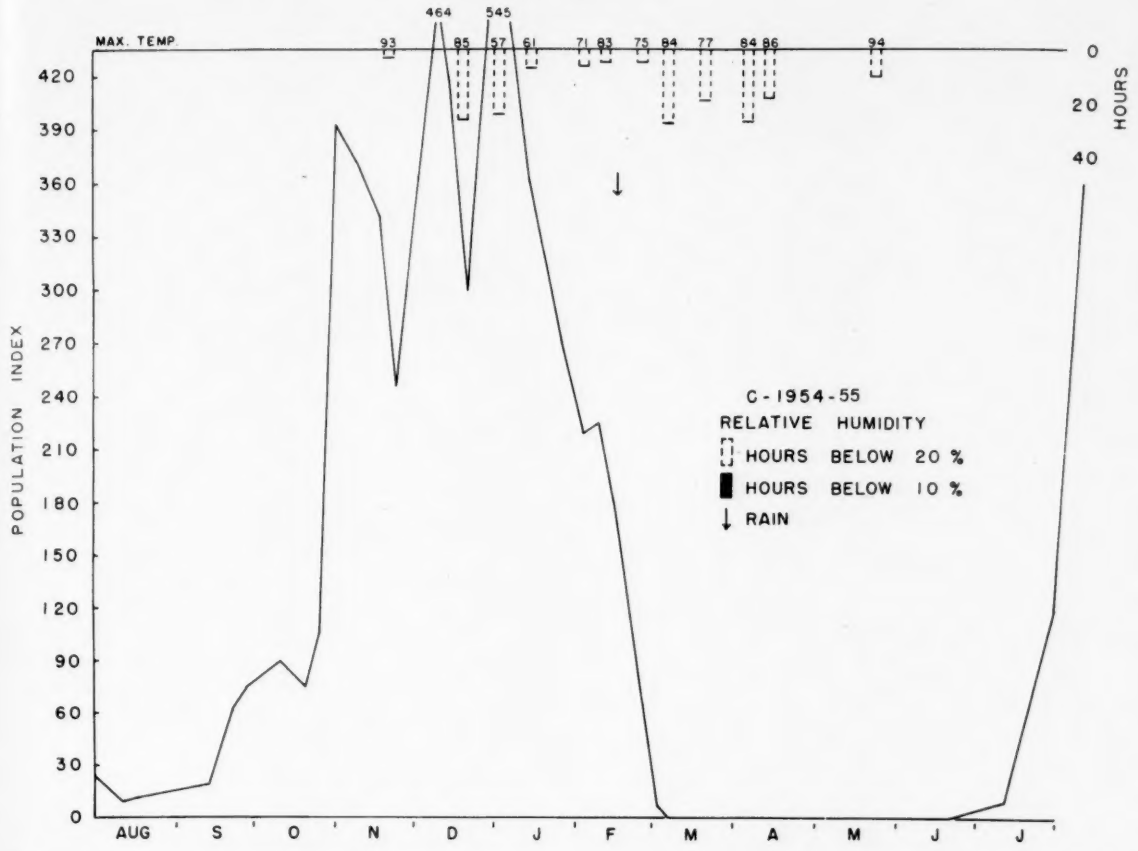


FIG. 2. A, B, and C.—Citrus red mite population trends on lemon trees in untreated plots in the Lambert orchard (Orange Co.) from August, 1952, to August, 1955. Bar graphs show periods of low humidity and maximum temperatures occurring during these periods.

decline during the last of May and through June, which is the time when the spring growth reaches maturity. The magnitude of the populations during the period from November, 1954, to January, 1955, at Los Posas (fig. 1, C) and at Lambert (fig. 2, C) indicates that the foliage and fruit during this period of minimum growth would support high mite populations if other factors were favorable. There was a marked decline in populations, however, some time between October and March each year in the Los Posas (fig. 1), Utt Development (fig. 3), and Hardison orchards (fig. 4), and during this period of 1952–53 and 1954–55 in the Lambert orchard (fig. 2, A and C), indicating that the mature growth was not sufficiently suitable to maintain populations if other factors were unfavorable. Where high mite populations had existed for an extended period on the mature growth, the feeding of the mites may have produced the unfavorable conditions which caused the population decline at Lambert during January and February in 1955 (fig. 2, C), and perhaps during one or more years in the Utt Development orchard (fig. 3); however, weather conditions appeared to be more frequently the important factor limiting the duration of high populations. In order to appreciate the variable influence of weather from one locality to another and from one year to another, a brief discussion of the factors influencing local weather conditions seems advisable.

Citrus districts of the coastal plain have a minimum daily fluctuation in temperature because, during the middle of the day, both the prevailing westerly winds and the sea breezes bring humid, cool air from the ocean. The Utt Development orchard is representative of districts having this coastal climate. The influence of the sea breezes may reach 10 to 15 miles inland unless the breezes are restricted by coastal hills. Districts not reached by sea breezes tend to have a more continental climate, which is characterized by maximum daily fluctuations of temperature and humidity. These districts are represented in this study by the Hardison orchard. Between these two situations, the effects of sea breezes on mites and/or their host vary from day to day and from season to season, depending on the extent and magnitude of the sea breezes as they are influenced by prevailing winds. The Los Posas and Lambert orchards represent districts which may be subject daily to both coastal and continental weather influences.

Hot or dry or hot and dry weather conditions may occur when northeast winds bring dry desert air to all citrus districts, but the extent and duration of these extremes may be modified by prevailing winds and sea

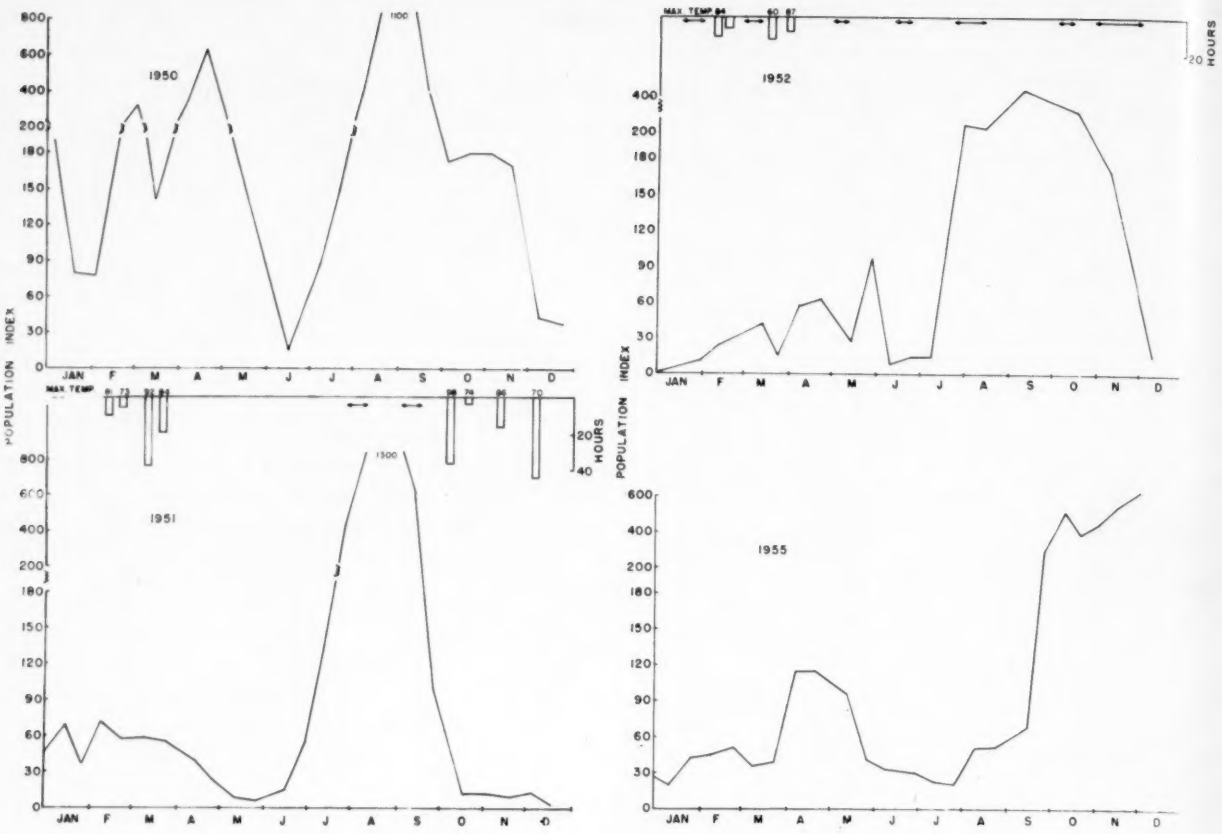


FIG. 3.—Citrus red mite population trends on lemon trees in untreated plots in the Utt Development orchard (Ventura Co.). Bar graphs show the number of hours during the week that humidity was below 20%.

breezes. As the adverse effect of such weather on mite populations is dependent on the duration and magnitude of the extremes, the influence of these winds on mites is often less in the more coastal districts. Although the occurrence of these extremes in weather are irregular, they are important factors influencing seasonal mite population cycles.

The mite populations in the Los Posas orchard were greatly influenced by temperature and humidity extremes. High temperatures and low humidities, associated with desert winds, caused important population reductions during October of 1952 and 1953. Each year, low humidities during the winter, when there was a minimum of new growth, appeared to be the cause of population reduction to such a low point that reinfestation did not occur until the following August.

A period of unusually hot weather occurred during the first part of September, 1955. At the Los Posas orchard, maximum temperatures above 100° F. were recorded on 4 successive days with a high of 107°; however, the relative humidity did not go below 28%. The rapid increase in mite populations after the middle of September indicated that a period of high temperature without low humidity had but limited adverse effect on mite populations when it occurred during the early part of a new growth cycle.

In the Lambert orchard the fall growth cycle provided conditions favorable for the rapid development of mite populations during September and October of the 1952 and 1954 season (fig. 2, A–C). As in the Los Posas orchard, the period of high temperature and low humidity accompanied by winds the last of October, 1952, and the last of September, 1953, caused a sharp decline in population density. In neither orchard did the populations return to the high, late fall peaks which characterized the 1954 season. This suggests that a combination of high temperature and low humidity not only has a direct adverse effect on mite populations, but also in some way changes the new growth so that it is no longer as favorable for mite development as growth which has not been exposed to such temperature and humidity extremes.

In contrast to the low populations found at the Los Posas orchard during June and July of each year, were the moderate to high population peaks found in the Lambert orchard during these months. Although there were not many hours in which the humidity was below 20% during June and July in either grove, when minimum humidities were averaged on a weekly basis it was found that the averages were about 10% lower at the Los Posas orchard than at the Lambert orchard; there were thus many daytime hours during the week when humidities were below 40%. This may be sufficient to account for the difference in general population trends in the two orchards.

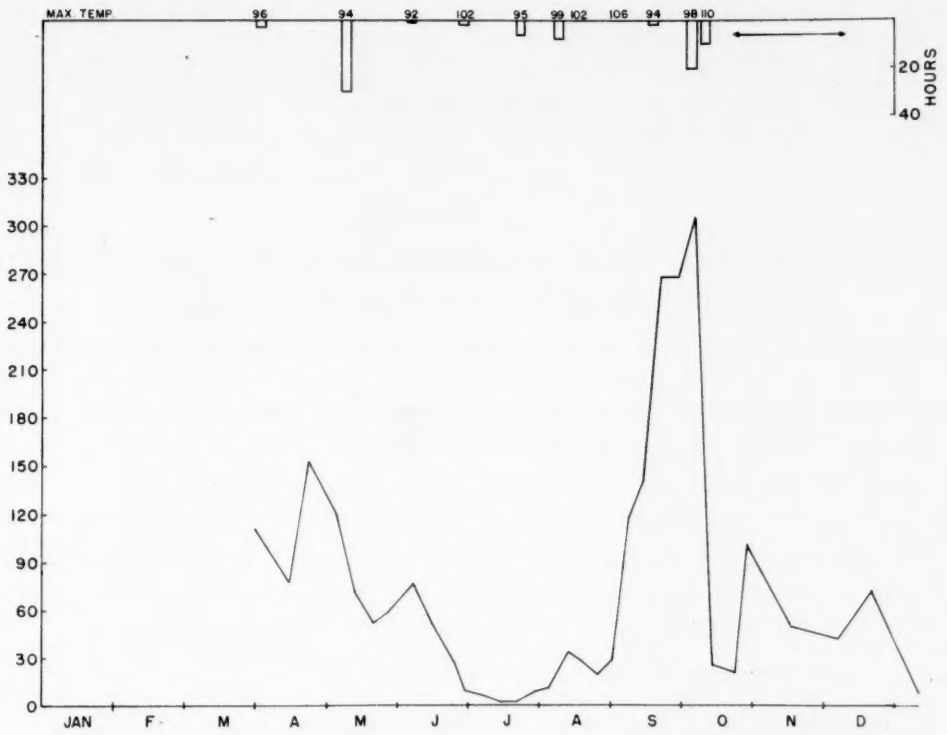


FIG. 4.—Citrus red mite population trends on lemon trees in untreated plots in the Hardison orchard (Ventura Co.) during 1950. Bar graphs show the number of hours during the week that relative humidity was below 20%.

Studies during 1950, 1951, and 1952 in the Utt Development orchard, near the coast, showed that mite populations increased very rapidly the last of July and reached the highest peaks in August and September. Weather extremes of sufficient magnitude to kill most of the mites or make growth unfavorable for mite development were rather infrequent in this district, and mite populations persisted throughout most of the year. Short periods of humidities below 20% were recorded during February and March of 1951 and 1952. These limited periods of adverse weather, together with the relatively cool daytime temperatures and minimum of new growth, probably accounted for the moderate rather than high populations during the winter and spring months of these years.

The Hardison orchard represents conditions more interior than those of the other three orchards. The population curve (fig. 4) indicates relatively short fall and spring population peaks. Predators were more abundant in this orchard than in the other orchard studied and were a major contributing factor limiting the height and particularly the duration of the population peaks. Further studies in this area are necessary to evaluate adequately the degree that the several factors modify the general seasonal population trends.

SUMMARY AND CONCLUSIONS.—Populations of the citrus red mite, *Metatetranychus citri* (McG.), were evaluated at weekly intervals over a 3-year period in untreated plots in four lemon groves. Continuous temperature and humidity records were also obtained in each grove. The trends in mite population were correlated with seasonal growth cycles of the tree and with periods of high temperature and low humidity. It was found that citrus red mite populations on lemon trees tend to be highest during periods when there is maximum amount of new foliage growth. Lemons, however, produce sufficient new growth to sustain relatively high populations most of the year if weather conditions are favorable. Low humidity and moderate to high temperatures were unfavorable for mite development, and low humidities accompanied by high temperatures and winds appeared to make new plant growth less favorable for mite population development. Temperature and humidity extremes were not of sufficient frequency in orchards near the coast to keep populations low for any extended period, whereas in an orchard more interior and under more continental weather influence, longer periods of the year were unfavorable for mite development.

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Correlation of Spray Treatments with Citrus Red Mite Population Trends on Lemon Trees in Southern California[1]

L. R. JEPPSON, M. J. JESSER, and J. O. COMPLIN, *University of California Citrus Experiment Station, Riverside*

In connection with studies on population trends of the citrus red mite, *Metatetranychus citri* (McG.), on lemon trees in the Los Posas orchard at Somis in Ventura Co. and the Lambert orchard at Tustin in Orange Co. (Jeppson *et al.* 1957), treatments were applied to replicated plots according to outlined schedules. These experiments were designed to ascertain when treatments should be applied in relation to population trends to obtain the most effective control. In similar studies on Valencia orange trees, Jeppson *et al.* (1953) had found that two treatments a year, applied at the beginning of each of the two growth cycles, namely, in March and September, resulted in the most effective annual mite control. Because lemon trees produce new growth most of the year, it seemed that the results of studies on oranges might not apply to lemons. The present studies were therefore undertaken.

MATERIALS AND METHODS.—Treatments were applied to three replicates of 4-tree plots, according to the following schedules:

Los Posas Orchard

A. Untreated
B. August and February
C. August and March
D. September and March
E. September and April
F. October and March
G. October and April
H. October and May
I. October and June
J. November and May
K. December and June
L. January and July
M. October
N. As needed
O. August and as needed

Lambert Orchard

A. Untreated
B. August and February
C. August and March
D. August and as needed
E. September and March
F. September and April
G. September and May
H. October and March
I. October and April
J. October and June
K. November and May
L. December and June
M. January and July
N. As needed

Spray applications were made during the first week of the months scheduled. Those made in August through January consisted of $1\frac{3}{4}$ gallons of California grade light-medium petroleum oil per 100 gallons of spray. Those made in February through July consisted of 1 pound of a 50% ovex wettable-powder formulation. The oil was used to provide control of scale insects as well as mites. Applications were made as full-coverage sprays by means of a conventional high-pressure spray equipment employing a reciprocating pump and manually operated spray guns. The citrus red mite populations were evaluated each week in each plot as described in a preceding paper (Jeppson *et al.* 1957).

RESULTS.—*Los Posas Orchard.*—The effects of spray applications made according to the schedules shown above, on citrus red mite population trends in the Los Posas orchard from August, 1952, to November, 1955, are reported in figure 1. In treated plots the degree of annual control obtained by two spray applications a year was dependent on the time of treatment in relation to the favorable period for population increase. Applications made the first week in August prevented the fall mite build-up (fig. 1, B and C), and those made the first week in September checked the mite population development before serious plant injury occurred (fig. 1, D and E). February through May applications all resulted in satisfactory control of the spring populations (fig. 1, B–H). Therefore, two applications, one made in August or September, and the other made during the period from February to May, resulted in effective citrus red mite control during the 3 years of study in this orchard. Applications at other times of year were less satisfactory, and treatments in January and July (fig. 1, L) resulted in little reduction in total mite population throughout the year as compared with the untreated trees.

Lambert Orchard.—The effects of spray applications made according to the schedules shown above, on citrus red mite population trends in the Lambert orchard from August, 1952, to August, 1955, are reported in figure 2.

August and September treatments in 1952 failed to prevent injurious infestations from occurring during October and November (fig. 2, B–G). In initiating the experiments, plots scheduled for treatment at a later date provided a large reservoir for mite migration into the comparatively few plots that were treated in August and September. Because of the relatively small plot size, migration into these plots from adjacent untreated trees no doubt accounts for ineffective control obtained from the August and September treatments in 1952.

Mite populations during 1952, when excessive migration from adjacent heavily infested trees was possible, were maintained at a lower level during the winter and spring (December through June), as a result of applications made in October or November, than as a result of applications made in August or September (fig. 2; compare B–G with H–K); however, August and September

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treatments in 1953 and 1954 prevented development of high mite populations through the winter months. Treatments made February to June failed in at least 1 of the 3 years to keep populations under control until the following September (fig. 2, E–G).

Mite populations were high on untreated plots in at least 1 of the 3 years of this study, in the fall, in the spring, and in July and August (fig. 2, A). Treatment scheduling must account for all three potentialities even though they do not occur every year. More critical timing appears to be necessary to control the citrus red mite by two spray applications per year in the Lambert orchard than in the Los Posas orchard (compare figs. 1 and 2).

August applications did not keep mite populations low

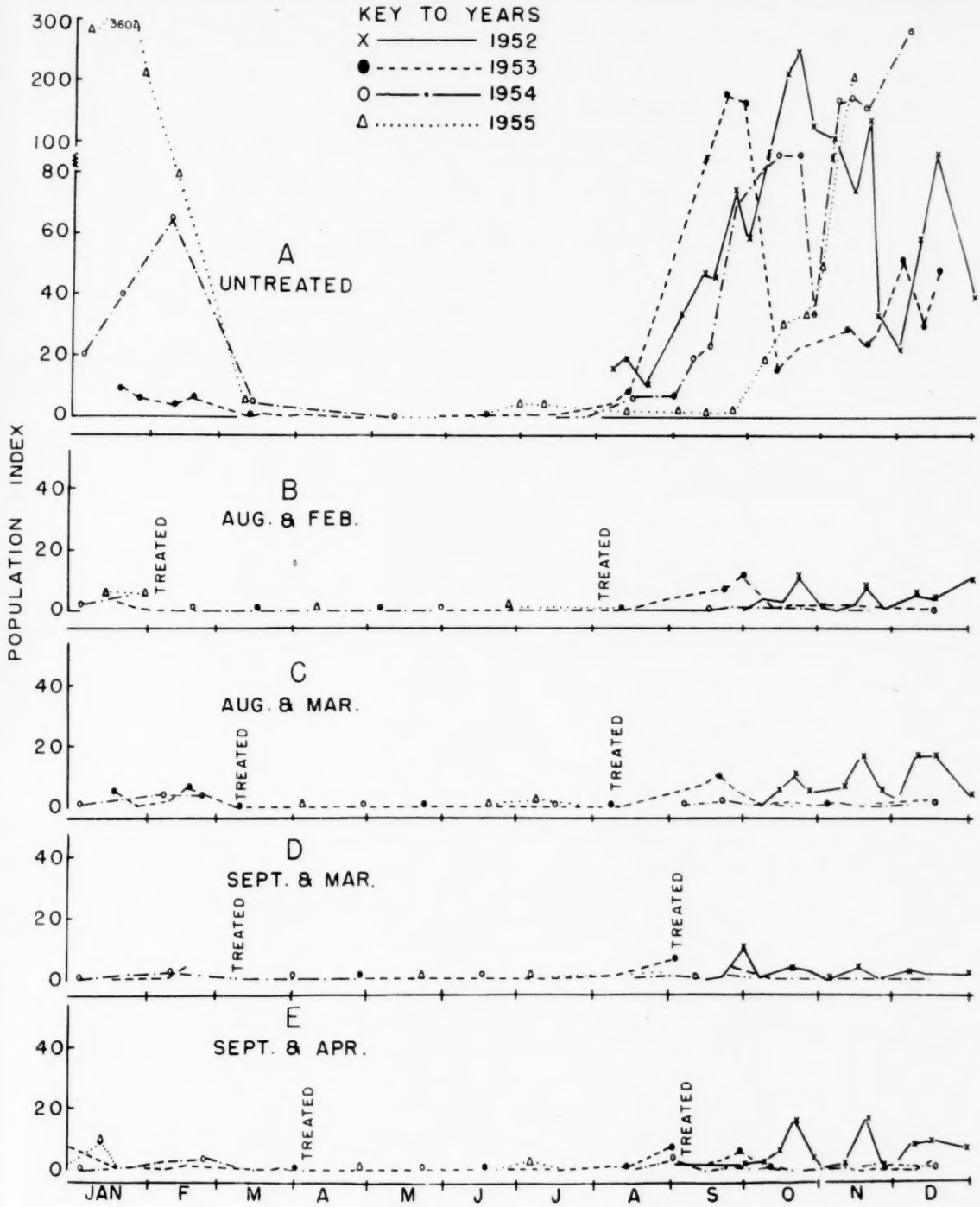

FIG. 1, PART 1—Citrus red mite population trends from August, 1952, to November, 1955, in plots in the Los Posas orchard sprayed according to indicated timing schedule.

beyond February, 1954 (fig. 2, D), and February-treated trees needed to be re-treated before August in both 1953 and 1955 (fig. 2, B). Plots treated in March, April, and May were moderately infested before September in at least 1 of the 3 years (fig. 2, E–G). Treatments in March, April, May, or June failed to keep populations under control until the following October (fig. 2, H–K). Treatments applied in August and March (fig. 2, C) resulted in low annual mite populations except during the first year (1952), when there was migration from adjoining plots;

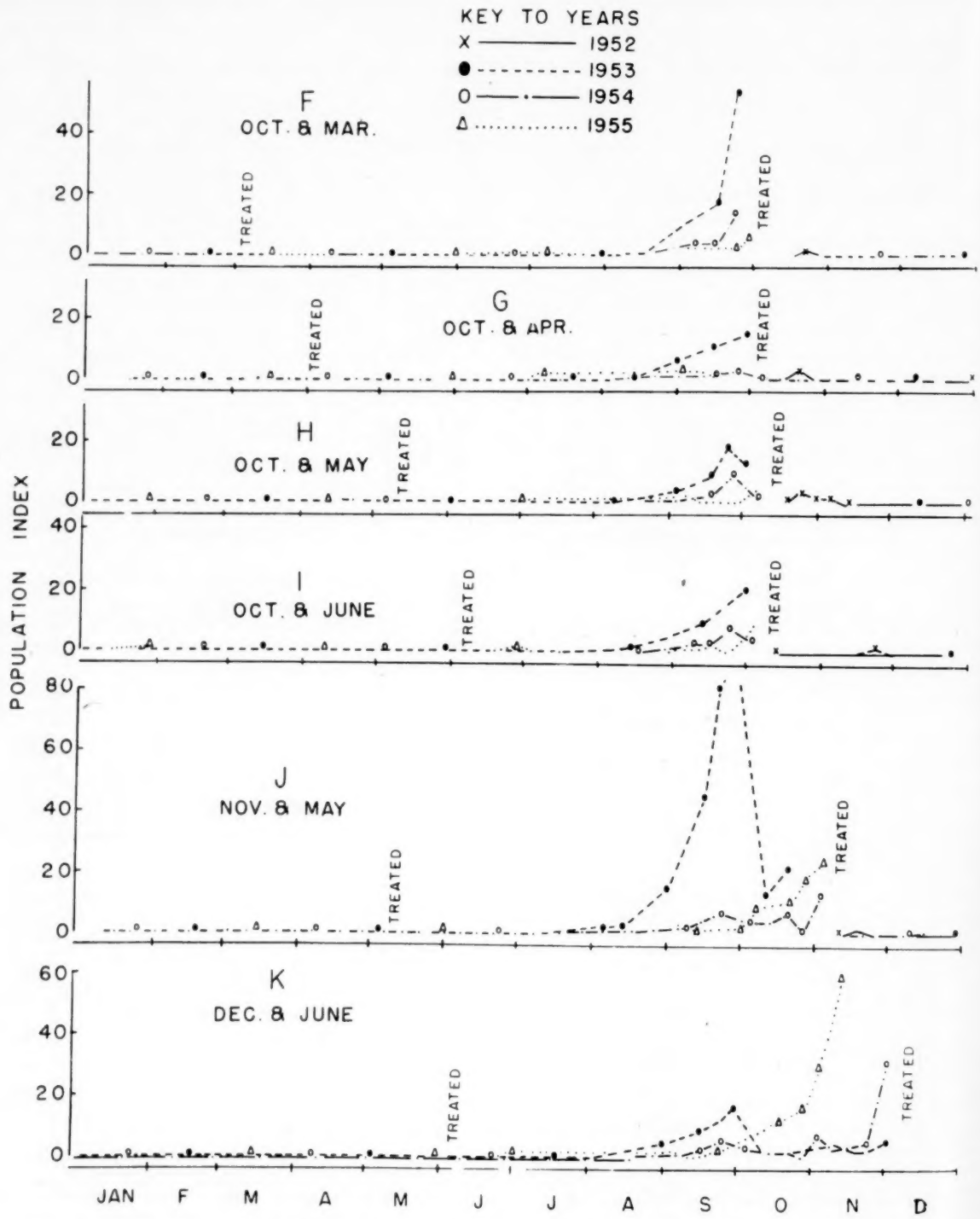

FIG. 1, PART 2—Citrus red mite population trends from August, 1952, to November, 1955, in plots in the Los Posas orchard sprayed according to indicated timing schedule.

however, plots treated in March on the March-September schedule (fig. 2, E) had a moderate population during July, 1954.

January and July applications resulted in effective control throughout all 3 years (fig. 2, M). Because of inclement weather conditions, January is often not a feasible time to make applications. It will be noted, however that treatments made in October and November effectively controlled the populations until May and June (fig. 2, J–L). As populations each year were on the decline in untreated plots during this period (fig. 2, A) it is unlikely that a major increase would occur at this time. It appears, therefore, that orchards having weather conditions similar to those of the Lambert orchard might be treated in October or November with petroleum oil, and again in July with either an oil spray or a good non-oil acaricide. According to this study the second-best schedule to consider includes applications in August and March (fig. 2, C).

SUMMARY AND CONCLUSIONS.—Populations of the

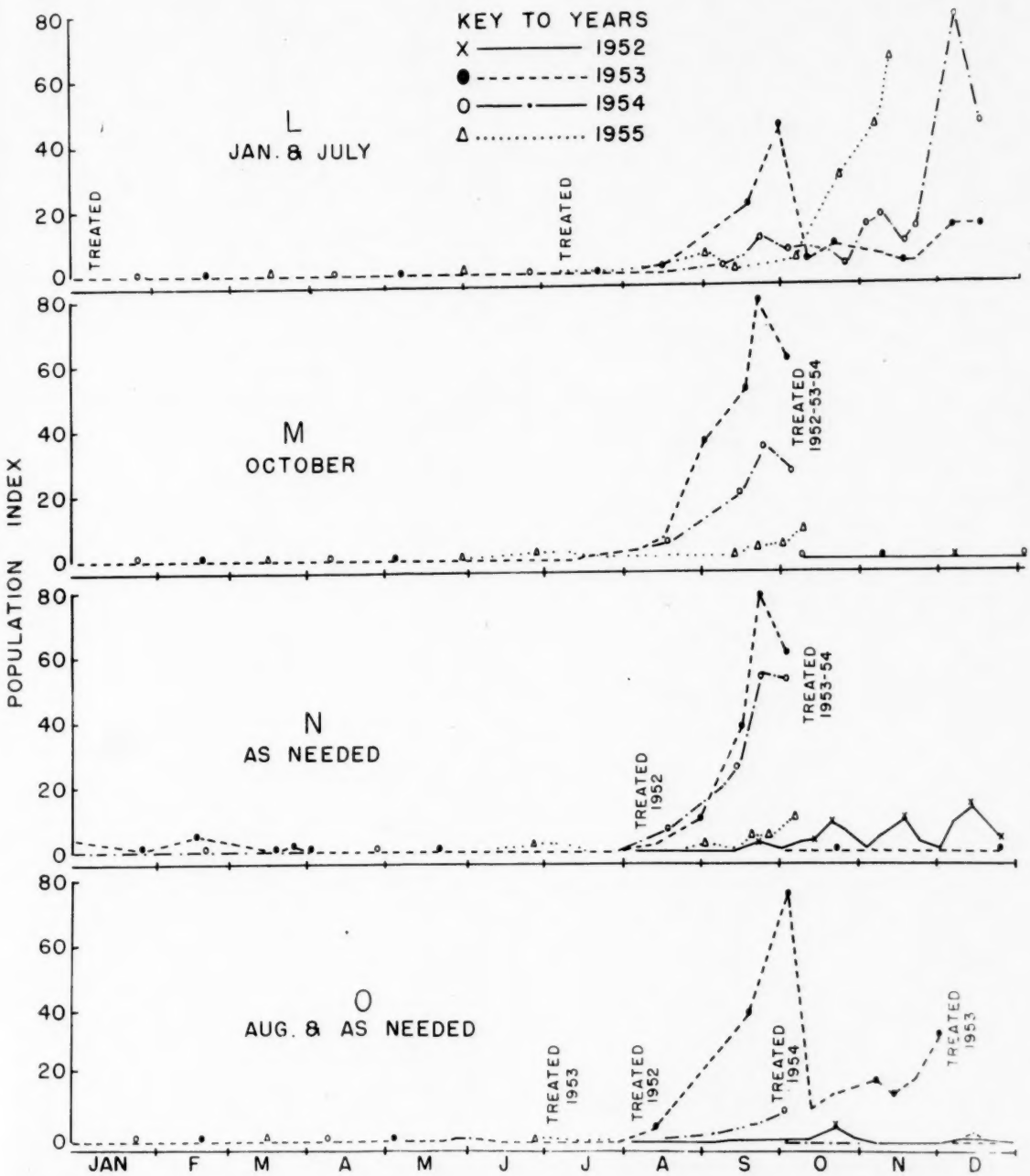

FIG. 1, PART 3—Citrus red mite population trends from August, 1952, to November, 1955, in plots in the Los Posas orchard sprayed according to indicated timing schedule.

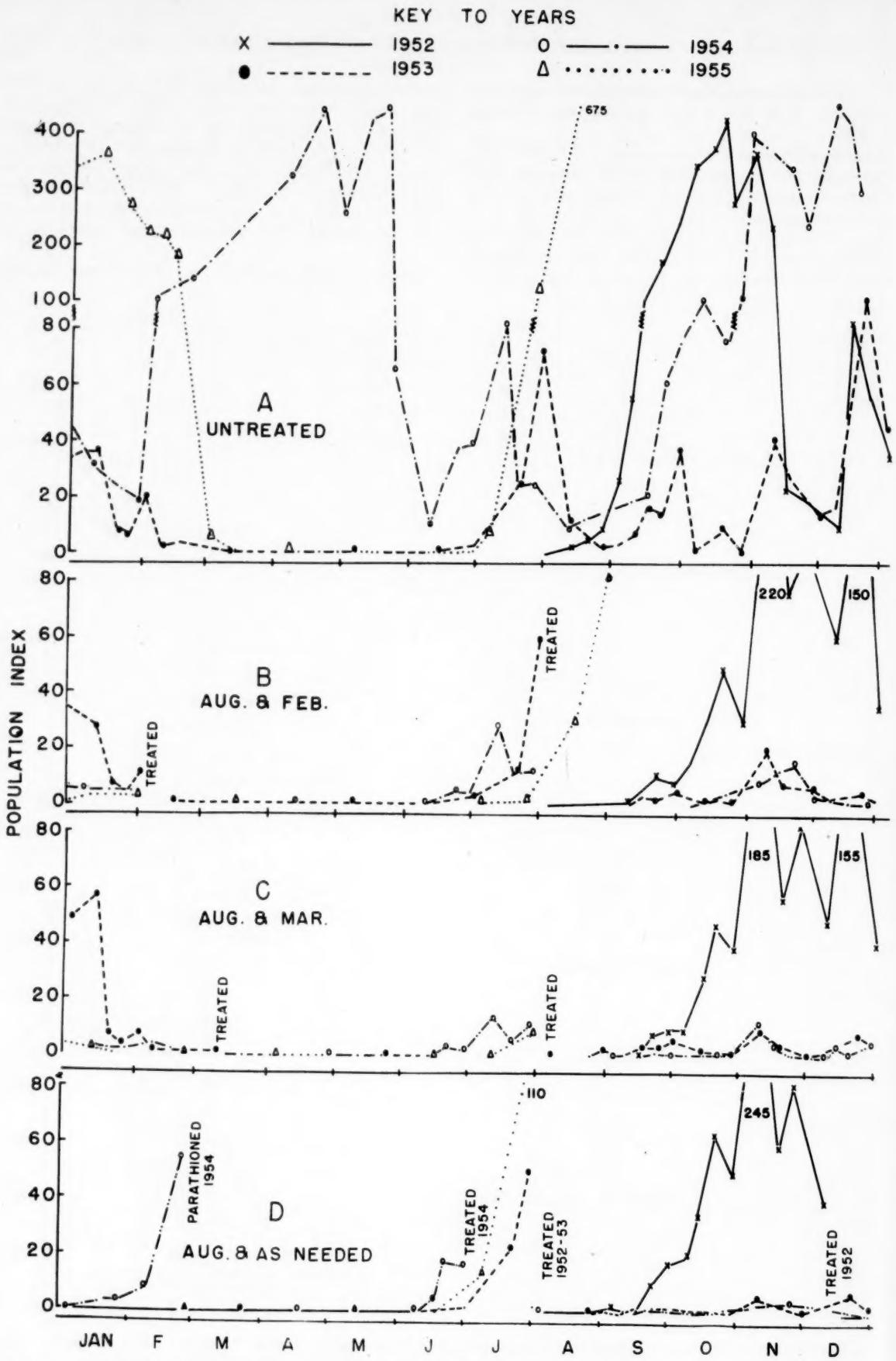

FIG. 2, PART 1—Citrus red mite population trends from August, 1952, to August, 1955, in plots in the Lambert orchard sprayed according to indicated timing schedule.

citrus red mite, *Metatetranychus citri* (McG.), were evaluated at weekly intervals on trees in two lemon groves which were treated semi-annually according to 14 or 15 different treatment schedules. The population trends that resulted from each treatment schedule were correlated with population trends on untreated trees.

The most effective schedule for control of citrus red mite on lemon trees in southern California was obtained when treatments were made at the beginning of major upward trends in population. Where orchards were so located that weather conditions were unfavorable for summer population peaks, treatments applied in August and

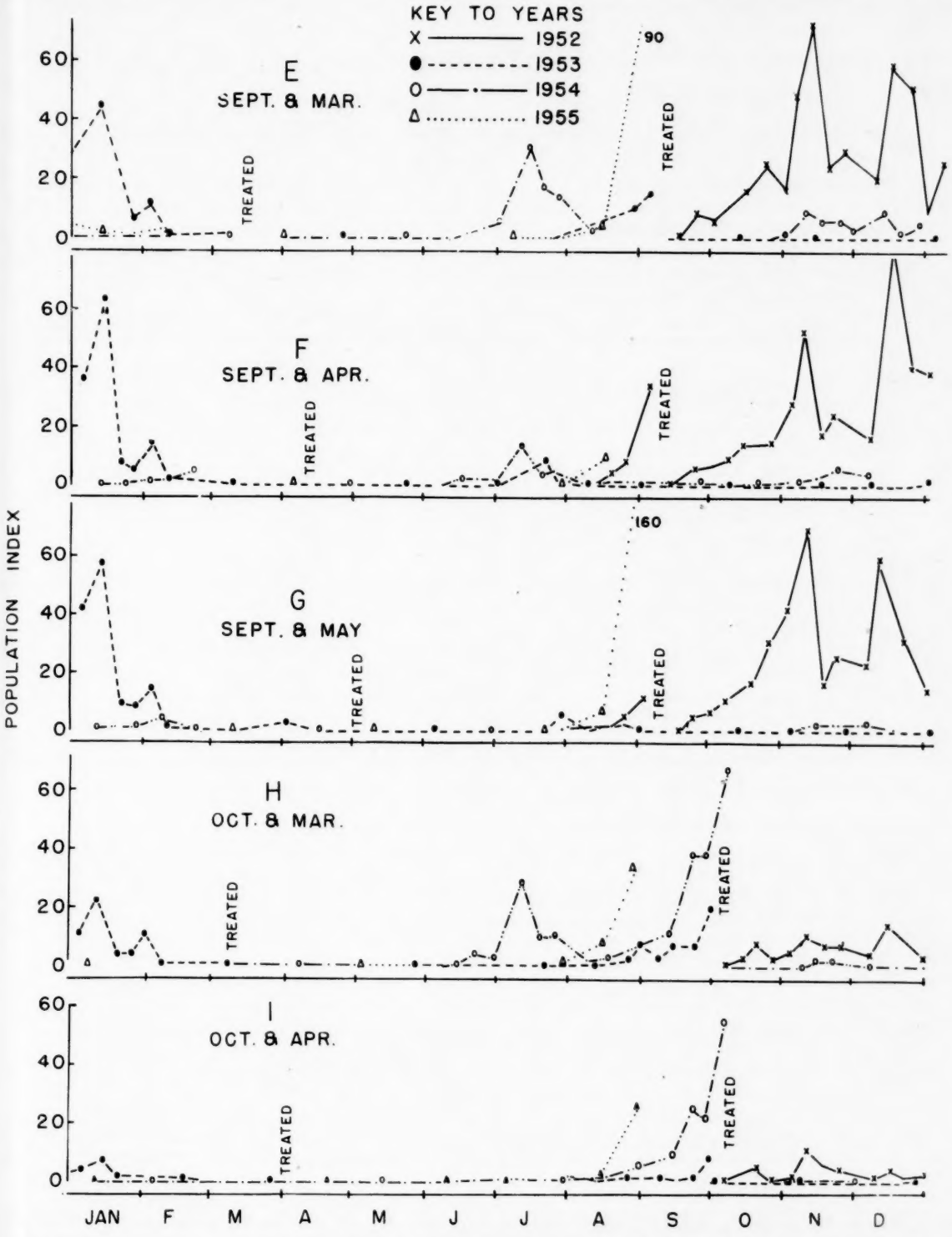

FIG. 2, PART 2—Citrus red mite population trends from August, 1952, to August, 1955, in plots in the Lambert orchard sprayed according to indicated timing schedule.

again in the spring (February through May) resulted in effective annual mite control. Where spring and summer weather conditions were not sufficiently severe to prevent moderate to high summer populations, a schedule consisting of treatments in October or November, and again in the latter part of June or July, is suggested. Orchards located on the coastal plain, such as the Utt Development orchard, where mite populations start to develop in June (Jeppson *et al.* 1957, fig. 3), should consider a June and October or June and November treatment schedule. These schedule suggestions are based entirely on most effective citrus red mite control. The final programming

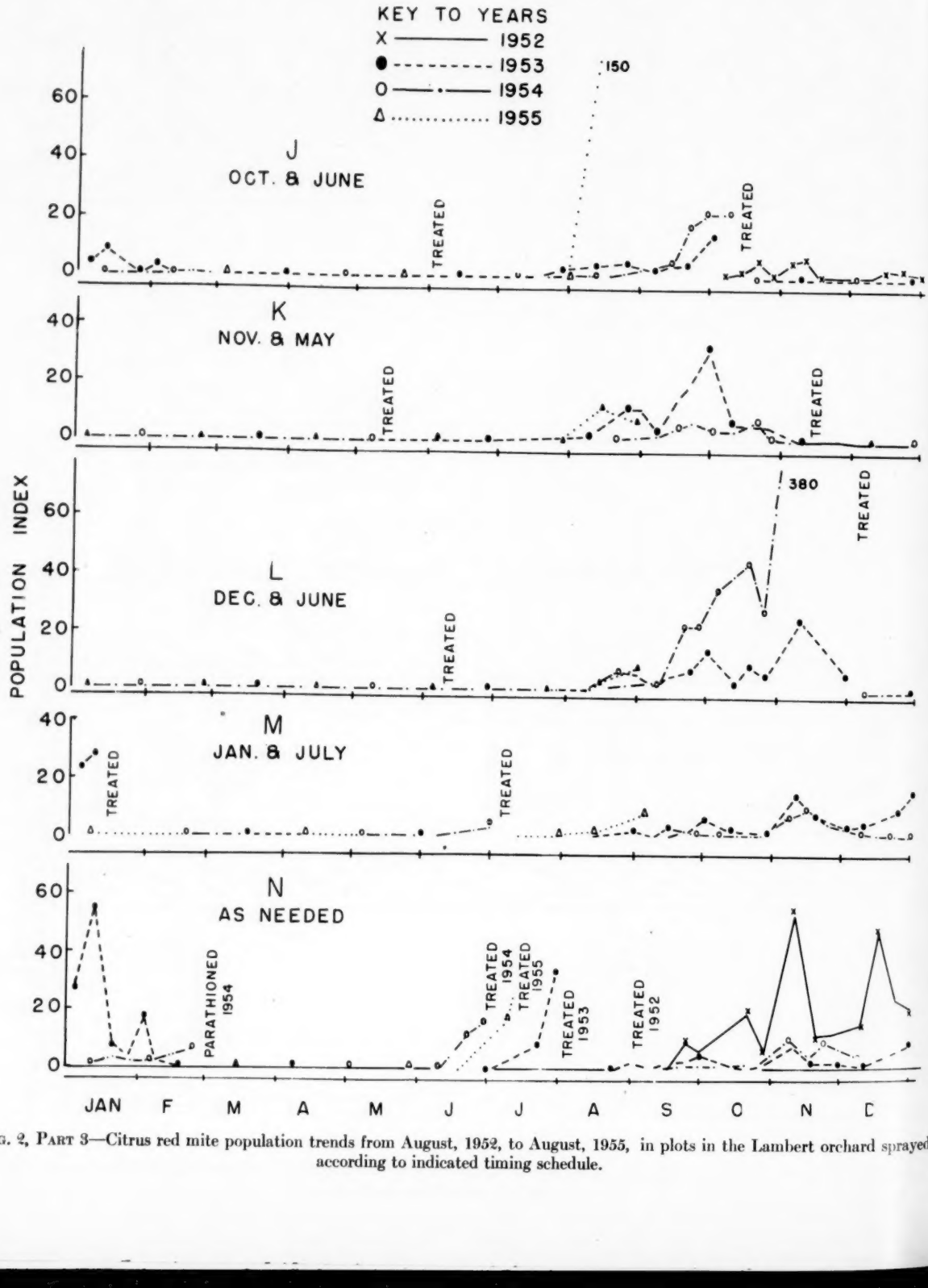

FIG. 2, PART 3—Citrus red mite population trends from August, 1952, to August, 1955, in plots in the Lambert orchard sprayed according to indicated timing schedule.

should take into consideration control of other pests, as well as fruit quality and good orchard management.

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Effectiveness of Two New Phosphate Insecticides for Control of Mites Injurious to Citrus in California[1]

L. R. JEPPSON, M. J. JESSER, and J. O. COMPLIN, *University of California Citrus Experiment Station, Riverside*

Acaricides currently available for control of the citrus red mite, *Metatetranychus citri* (McG.), have some undesirable characteristics which restrict their use or cause failure to give adequate control under certain conditions. Additional materials for effective control of this mite are desirable.

In initial field screening tests, applications of 2,3-*p*-dioxandiethiol *S,S*-bis(*O,O*-diethyl phosphorodithioate), or Hercules AC-528 (Hercules Powder Company, Wilmington, Delware), and of *S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate, or Trithion (Stauffer Chemical Company, Mountain View, California) resulted in effective control of the citrus red mite. In this paper results obtained with Compound 528 and Trithion used against the citrus red mite, the six-spotted mite, *Eotetranychus sexmaculatus* (Riley), the Yuma mite, *Eotetranychus yumensis* (McG.), the citrus bud mite, *Aceria sheldoni* (Ewing), and the citrus flat mite, *Brevipalpus lewisi* McG., are compared with those obtained with standard materials currently used for control of each of these citrus mites.

MATERIALS AND METHODS.—Conventional sprays were applied by means of orchard spray rigs equipped with high-pressure reciprocating-type pumps and manually operated spray guns. A tower was used where adequate coverage was not readily obtained by spraying from the ground. Sprays were applied at 1,500 to 2,000 gallons per acre, the rate depending on the size of the trees. Boom-spray applications were made with an oscillating boom sprayer equipped with a high-pressure centrifugal pump. Spray-blower applications were made by means of a fish-tail-type, high-air-volume blower equipped with spray pump capable of discharging spray into the air stream in the fishtail under 300 pounds pressure.

Plots treated for citrus red mite control consisted of eight trees each, with adequate buffer rows to allow for spray drift. Each treatment was applied to two plots in each grove. Citrus red mite populations were evaluated by counting the adult mites on 32 leaves on each of the eight trees in each plot, as described by Jeppson (1951). Results are reported as average number of mites per tree (32-leaf sample). The period of toxicity of spray residues to mites was evaluated by picking eight fruits per treatment at intervals after applications of the spray. The fruit was brought into the laboratory, and 25 adult citrus red mites were placed on each of the eight fruits. The net percentage mortality was obtained after an exposure of 48 hours.

To evaluate populations of the citrus flat mite, two 1-inch-square areas were examined on each of eight fruits per tree, eight trees per plot, by means of a linen counter. Results are reported as average number of mites per square inch of fruit surface. Evaluations of populations of the citrus bud mite were made by examining five buds on each of 15 new-growth terminals from each of eight trees per plot. Results are reported as percentages of buds infested.

RESULTS.—The rates of repopulation of citrus red mites in six lemon groves and in two orange groves treated with Compound 528, Trithion, and standard acaricides applied by conventional spray equipment are reported in table 1. Results of boom-spray applications are reported in table 2, and results of spray-blower applications are reported in table 3. The relationships between the number of days of weathering in the field and the toxicity of spray residues of Compound 528 and of Trithion to adult citrus red mite on three citrus varieties are shown in table 4. The Navel orange and lemon fruits were picked at the same time that samples were taken for chemical residue analysis. Results of chemical residue analysis show that Compound 528 had a persisting half life of 95 days on navel oranges and 85 days on lemons. Trithion had a half life of 42 days on navel oranges and 22 days on lemons. The complete results will be reported later. Results of applications of Compound 528 and of Trithion for control of citrus bud mite and of citrus flat mite are reported in tables 5 and 6, respectively.

DISCUSSION.—This study indicates that Compound 528 and Trithion are effective treatments for control of citrus red mite on citrus in California. Spray applications of either of these materials resulted in more effective control of this mite than similar applications of light-medium petroleum oil or of an extensively used combination of ovex and Aramite (tables 1 and 2). The relative effectiveness of either Compound 528 or Trithion and demeton differed in the several tests. It appears, however, that at least twice as much of these materials as of demeton is required to control the citrus red mite (tables 1 and 2). Boom-spray applications of Compound 528 and Trithion were more effective than similar applications of demeton, whereas conventional applications of demeton were more comparable to these materials. Further evaluation is necessary to establish the reason for these differences.

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Table 1.—Relative effectiveness of Compound 528, Trithion, and standard acaricides when applied to lemon and orange trees by conventional spray equipment for control of citrus red mite.

Formulation[a]	Actual Toxicant (Oz. per 100 Gal.)	Average Number of Live Adult Mites per 32-Leaf Sample on Days After Treatment Indicated[b]			
On Lemon Trees					
A. Riverside County Grove (Treated June 18, 1954)		22	63	79	123
528 25% EC	4	0.0	0.3	7.0	4.6
528 25% WP	4	3.4	1.8	77.6	135.0
528 25% WP	8	0.1	0.0	12.5	28.0
Demeton 21.2% EC	2	0.0	0.3	5.0	17.5
B. Orange County Grove (Treated October 15, 1954)		234	279		
528 25% WP	4	1.0	53.0		
528 25% WP	8	1.0	5.0		
Trithion 25% EC	2	10.0	140.0		
Trithion 25% EC	3	1.0	9.0		
Demeton 21.2% EC	1	1.0	98.0		
Demeton 21.2% EC	2	0.0	45.0		
C. San Diego County Grove (Treated October 17, 1955)		226	240	275	
528 25% EC	4	0.2	9.0	177.0	
528 25% EC	8	0.0	0.4	29.0	
Trithion 25% WP	4	1.2	12.0	217.0	
Trithion 25% WP	8	0.0	0.0	4.6	
Demeton 26.1% EC	2	0.0	0.0	3.8	
D. Riverside County Grove (Treated November 4, 1955)		193	219	241	256
528 25% EC	4	0.0	2.9	2.5	14.0
528 25% EC	8	0.0	27.8	5.5	22.5
528 25% WP	4	0.0	8.2	0.3	12.3
528 25% WP	8	0.0	7.9	0.9	4.5
Trithion 50% EC	4	0.4	20.9	11.0	102.5
Trithion 50% EC	8	0.3	10.0	12.1	53.0
Trithion 25% WP	4	0.2	20.0	5.6	49.5
Trithion 25% WP	8	0.7	6.0	11.3	30.5
Demeton 26.1% EC	2	1.0	9.9	1.5	15.5
Demeton 26.1% EC	3	9.5	10.4	1.6	8.0
E. Ventura County Grove (Treated February 8, 1956)		82	109	126	139	166
528 25% EC	6	1.5	4.9	57.0	75.8	RT
Trithion 25% WP	6	0.0	0.0	3.2	1.6	24.5
Trithion 25% EC	6	0.0	0.0	0.6	0.3	11.5
Demeton 26.1% EC	2	0.1	0.1	10.0	2.2	52.0
Ovex 50% WP	8	3.0	9.7	115.0	RT	
Aramite 15% WP	1.8					
F. Ventura County Grove (Treated February 16, 1956)		25	54	74	118	171
528 25% EC	6	0.0	0.0	0.0	4.6	108.0
Trithion 25% WP	6	0.0	0.0	0.0	0.4	205.0
Trithion 50.1% WP	12	0.0	0.0	11.0	0.1	12.0
Ovex 50% WP	8	0.4	11.0	83.0	RT	
Aramite 15% WP	1.8					
On Orange Trees					
G. Riverside County Grove (Treated March 22, 1955)		93	109	164	
528 25% EC	4	0.0	0.2	0.8	
	8	0.0	1.3	3.5	
	12	0.0	0.0	2.8	
Trithion 25% WP	3	0.2	0.1	2.5	
	4	0.0	0.0	4.5	
	6	0.1	0.0	2.5	
	8	0.0	0.0	2.5	
Demeton 21.2% EC	1.5	0.0	0.0	2.0	
	3.0	0.0	0.0	2.2	
Genite 25% EC	16	0.0	2.0	42.0	
H. Riverside County Grove (Treated April 19, 1955)		34	59	90	128
528 25% EC	8	0.0	0.0	0.2	1.0
528 25% WP	8	0.0	0.3	0.0	0.0
Trithion 25% WP	2	1.5	2.5	0.7	40.0
	4	0.0	0.3	0.0	8.5
	8	0.0	0.5	0.0	8.5
Demeton 26.1% EC	2	0.0	0.0	0.0	7.0

[a] EC = emulsifiable concentrate; WP = wettable powder.
[b] RT = re-treated.

Table 2.—Relative effectiveness of Compound 528 and standard acaricide treatments when applied by boom-spray equipment to Valencia orange and lemon trees for control of citrus red mite.

Formulation[a]	Amount Actual Toxicant per 100 Gallons	Average Number of Live Adult Mites per 32-Leaf Sample on Days After Treatment Indicated[b]				
A. Lemon Grove, Ventura County (Treated October 14, 1955)		110	191	234	264	283
528 25 EC	4 oz.	0.0	0.0	7.2	6.5	39.0
Trithion 25% WP	4 oz.	0.0	0.0	0.0	0.3	17.0
Ovex 50% WP	4 oz.	90.0	RT			
Petroleum oil (light medium emulsive)	1.75 gals.	1.2	150.0	RT		
B. Lemon Grove, Riverside County (Treated November 11, 1955)		80	156	174	188	
528 25% EC	4 oz.	0.2	0.0	0.5	8.2	
Trithion 25% WP	4 oz.	0.0	0.0	1.5	11.5	
Demeton 26.1% EC	2 oz.	0.1	0.0	8.4	32.0	
Ovex 50% WP	4 oz.	8.0	20.0	RT		
Aramite 15% WP	2.4 oz.	7.1	23.0	RT		
C. Valencia Orange Grove, Ventura County (Treated February 21, 1956)		69	96	134	161	181
528 25% EC	5 oz.	0.0	3.0	7.0	26.0	17.0
Trithion 50% EC	5 oz.	0.0	0.0	0.0	3.0	23.0
Petroleum oil (light-medium emulsive)	1.33 gal.	13.0	28.0	70.0	RT	
D. Lemon Grove, Riverside County (Treated March 1, 1956)		102	137	162	172	
528 25% EC	4 oz.	0.3	1.5	25.0	100.0	
Trithion 25% DC	4 oz.	0.0	2.3	77.0	263.0	
Demeton 26.1 EC	2 oz.	0.1	94.0	RT		

[a] EC = emulsifiable concentrate; WP = wettable powder.
[b] RT = re-treated.

In many of the experiments where Compound 528 and Trithion were applied to orange trees at a range of dosages, effective control of citrus red mite was obtained at all dosages for the duration of the test. Results from one of these tests are reported in table 1, G. In orange groves, where the mites increased more rapidly (table 1, H), and in most of the evaluations on lemon trees (table 1, B–F), the higher the dosage of Trithion used, the more effective the control.

Table 3.—Relative effectiveness of Compound 528, Trithion, and Demeton when applied to citrus trees by sprayblower equipment at 600 gallons of spray per acre for control of citrus red mite.

Formulation[a]	Actual Toxicant (Pounds/Acre)	Average Number of Live Adult Mites per 32-Leaf Sample on Days After Treatment Indicated		
A. Lemon Grove, Riverside County (Treated July 15, 1954)		116	180	218
528 25% EC	4	0.2	1.0	17.0
Demeton 21.2% EC	1	2.5	0.5	62.0
B. Navel Orange Grove, San Bernardino County (Treated April 4, 1956)		72	107	126
528 50% EC	5	5.0	15.1	18.0
Trithion 50% EC	3.5	0.4	21.6	23.6
Demeton 26.1% EC	2	7.0	15.5	8.1

[a] EC = emulsifiable concentrate.

Table 4.—Toxicity of spray residues of Compound 528[a] and of Trithion[b] on citrus fruits to citrus red mite after various intervals of weathering in the field.

Actual Toxicant (Oz./100 Gal.)	Per Cent Mortality of Adult Mites on Fruits Picked at Indicated Days After Treatment							
Compound 528								
Valencia Orange Trees (Sprayed May 4, 1955)	14	28	48	65	93	114	136
8	100	97	100	99	94	73	57
Navel Orange Trees (Sprayed February 20, 1956)	17	24	38	53[c]	64	78	
12	100	98	97	84	70	34	
Lemon Trees (Sprayed March 19, 1956)	8	16	24	30	37	51	65	79	93
6	100	100	84	90	59	38	—	—	—
12	99	100	97	—	98	81	76	80	43
24	100	100	100	99	97	97	95	72	77
Trithion							
Valencia Orange Trees (Sprayed May 4, 1955)	28	48	65	93	114	136
8	100	98	95	87	86	75
Navel Orange Trees (Sprayed February 15, 1956)	44	51	62[c]	69	83	97
4	100	98	99	93	91	69
12	100	100	100	100	100	98
24	100	100	100	100	100	97
Lemon Trees (Sprayed March 7, 1956)	16	23	31	42	63	77
4	100	98	46	63	20	—
12	100	100	100	100	80	14
24	100	100	100	100	95	55
12[d]	100	100	100	100	94	69

[a] Emulsifiable concentrate formulations were used.
[b] Wettable powder formulations were used.
[c] Six inches of rain fell just prior to the taking of this sample.
[d] Emulsifiable concentrate formulation was used for this treatment.

In the June, 1954 conventional spray applications of Compound 528 in Riverside County, the emulsifiable concentrate formulation was more effective than the wettable powder formulation; however, results from the treatments of November 4, 1955, with the two formulations resulted in more nearly the same degree of control (table 1; compare A and C). In three of the four groves in which 4- and 8-ounce dosages of Compound 528 were compared (table 1, A–C), the high dosage resulted in the more effective control; but in the one test reported (table 1, D) and in other tests not reported because mite populations were not of sufficient magnitude to show differences between treatments, the results from the lower dosage resulted in effective control. Apparently, the higher dosage would be advisable only in groves where the mites are an unusually serious problem.

The persistence in toxicity of the spray residues of Compound 528 and Trithion appears to be an important factor in their effectiveness in controlling the citrus red mite. The residues of Compound 528 were more persistent on Valencia oranges sprayed May 4, 1955, than on navel oranges sprayed Febrary 20, 1956 (table 4); but this does not appear to be true for residues of Trithion (table 4). Six inches of rain were recorded just prior to the taking of the 53-day sample of Compound 528 and the 62-day Trithion sample of navel oranges. Considerable amounts of the surface residues of Compound 528 may have been removed at that time, although the rain did not appear to reduce the toxicity of the Trithion residues. The oranges were treated after the fruit had reached full size, whereas the lemons were growing while the residues were weathering. This probably accounts for the shorter interval of residue effectiveness and the lower half life of both of these materials on the lemon fruit (table 4).

Table 5.—Relative effectiveness of Compound 528, Trithion, Chlorobenzilate, and petroleum oil for control of citrus bud mite on lemons.

Formulation[a]	Amount Actual Toxicant per 100 Gal.	Per Cent Buds Infested with Bud Mite on Days After Treatment Indicated		
		27	63	100
528 25% EC	8 oz.	9	40	82
Trithion 25% WP	8 oz.	7	28	92
Chlorobenzilate 25% WP	4 oz.	0	0	18
Petroleum oil (light-medium emulsive)	1.75 gal.	0	6	35

[a] EC = emulsifiable concentrate; WP = wettable powder.

Applications of Compound 528 or of Trithion to three groves for control of the six-spotted mite, and to two groves for the control of the Yuma mite, resulted in effective control for the season or until treatment was necessary to control other pests.

Applications of Compound 528 or Trithion were not so effective as petroleum oil or Chlorobenzilate in the control of citrus bud mite (table 5), and were relatively ineffective against the citrus flat mite (table 6). No phytotoxic effects were observed to follow any of the spray applications herein reported, although leaf injury did occur on plots treated with Trithion in November, 1956.

SUMMARY.—The effectiveness of several dosages and formulations of Hercules AC-528 (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) and Trithion

Table 6.—Relative effectiveness of Compound 528, Trithion, and Chlorobenzilate for control of the citrus flat mite.

Formulation[a]	Actual Toxicant (Oz./100 Gal. Spray)	Average Number of Live Adult Mites Per 2 Sq. In. of Fruit Surface on Days After Treatment Indicated	
		48[b]	26[c]
528 25% WP	4	0.88	0.25
	8	0.88	0.38
Trithion 25% WP	4	1.75	—
	8	0.88	4.97
Chlorobenzilate 25% WP	4	0.00	0.13

[a] WP = wettable powder.
[b] Conventional spray applications.
[c] Boom-spray application.

(*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) were evaluated by conventional spray, boom-spray, and spray-blower applications for control of citrus red mite, *Metatetranychus citri* (McG.). The results indicate that these materials varied in their effectiveness according to the dosage applied and were generally more effective by all three methods of application than similar applications of the formulations of ovex or petroleum oil currently used for control of this mite. Trithion and Compound 528 also resulted in control of six-spotted mite, *Eotetranychus sexmaculatus* (Riley), and Yuma mite, *Eotetranychus yumensis* (McG.), but were ineffective for control of citrus bud mite, *Aceria sheldoni* (Ewing), and citrus flat mite, *Brevipalpus lewisi* McG.

Field residues were toxic to adult citrus red mite for a longer period of time on oranges than on lemons. Similar residues measured by chemical analysis showed a longer half life on navel oranges than on lemons.

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An Ecological Study of the Cigarette Beetle in Tobacco Storage Warehouses[1]

FRANK P. SIVIK,[2] JOSEPH N. TENHET,[3] and CARL D. DELAMAR[4]

The cigarette beetle, *Lasioderma serricorne* (F.), (Coleoptera: Anobiidae) is the most destructive insect pest found on stored tobacco. It is cosmopolitan in distribution, and is usually present at all times in some stages in tobacco storage warehouses. The cigarette beetle was known to be present in Egypt more than 3500 years ago (Alfieri 1931) and was first recorded as a pest of cured tobacco in North Carolina 70 years ago. The control, as well as the ecology, of this insect has been studied but most of the latter has been restricted to the laboratory. The studies reported in this paper were conducted during the summer of 1956 in tobacco storage warehouses, and a high cigarette grade of flue-cured tobacco of the 1953 crop was the only host food used for the various experiments.

This ecological study includes such factors as life history, proportion of sexes found in daily insect light trap collections, fecundity of the female, an endeavor to determine why the cigarette beetle flies upward, the percentage of beetles emerging from hogsheads, and some observations on mating.

LIFE HISTORY.—The life history study was undertaken to determine the number of days the cigarette beetle requires for development during the summer on flue-cured tobacco in a storage warehouse. Other workers have found the life cycle as follows:

	Duration of Period (Days)					
Worker	Egg	Larval	Pupal	Life Cycle	Host Foods	Conditions
Jones (1913)	6	50	12.5	68.5	Tobacco	Factory and Laboratory
Runner (199)	6–10	30–50	6–10	45–70	Tobacco	Factory and Laboratory
Back & Cotton (1927)	—	—	—	42–70	—	—
Powell (1931)	—	—	—	57–65	Tobacco	Laboratory
Bare *et al.* (1947)	7	35	8	—	Corn meal and yeast	Laboratory

In our experiments, the beetles were reared in the warehouse where the room temperature averaged 85° F. and the relative humidity was about 60%. Heat-treated, flue-cured tobacco, free of beetle life in all stages, was used for the beetle rearing. A larva was placed on a section of tobacco leaf in a small petri dish. Periodically, a drop of water was placed on the inside of the petri dish cover to keep the tobacco from drying. It was observed that of 113 eggs, 63% hatched and 25% became adults. The life cycle data (table 1) differs little from that of previous workers, although they are more comparable with the data of Bare *et al.* than with that of the others. Bare *et al.* did not use flue-cured tobacco, but a mixture of corn meal and dry yeast. According to Powell (1931), yeast is an ideal food which tends to shorten the life cycle of the beetle.

The egg is ovoid-elliptical, whitish, becoming opaque and dull in color just before hatching. The general external appearance is smooth, without reticulation except for a portion at the end from which the larva emerges, which is covered with numerous spines. The average length was 0.45 mm. and the width was 0.21 mm. The eggs were deposited singly in small folds and crevices of leaf tobacco, most often along the midrib. The time of most active egg-laying was in the early evening and during the night. The length of the incubation period was about 7 days.

The newly-hatched larva was less than 1 mm. in length and covered with fine hair. The head was yellowish, the body semi-transparent and whitish. The grub crawled short distances in search of food and ceased to migrate after beginning to feed. The insect damaged tobacco by eating small cylindrical galleries through the host material. Fine particles of tobacco, dust and frass adhered to the minute hairs on the body, often changing the appearance to a brownish color. This was especially noticeable in the last instar. When disturbed, the larva assumed a curled attitude, the abdomen being bent round almost double. The excrement was dark brown to black and the

[1] Research was conducted at Durham, North Carolina, by the Liggett and Myers Tobacco Co. in cooperation with the Agricultural Marketing Service, U. S. Dept. of Agriculture. Accepted for publication December 19, 1956.

[2] Formerly Entomologist, Liggett and Myers Tobacco Co. Present address: Stored-Products Insect Section, U. S. Dept. of Agriculture, P. O. Box 3425, Savannah, Ga.

[3] Entomologist in Charge, Stored Tobacco Insects Laboratory, U. S. Dept. of Agriculture, Richmond, Va.

[4] Chemical Engineer, Liggett and Myers Tobacco Co.

individual pellets were more or less pointed at both ends. The full-grown larva measured about 0.16 inch long. The average length of the larval period was about 40 days. After molting four to five times, the larva constructed a smooth-lined cell in which to rest. The insect passes the winter in the larval stage in a state of quiescence.

Pupation did not occur immediately after the cell had been constructed. The larva lay quiescent in a curved position within the cell and gradually took on the pupal form. The newly formed pupa was glossy white but gradually turned to a reddish-brown after a few days. Within the pupal cell, the pupa lay on its back with the legs, wings, eyes and other external features of the adult being readily apparent. The pupal skin frequently adhered to the tip of the abdomen for a short time after adult emergence had taken place. The pupal period was about 5 days.

The adult beetle is small, brownish in color and varies in size. Generally the females are larger than the males. They fly freely in the evening and during the night. The period of greatest activity is usually between 4:30 P.M. and midnight (Tenhet 1955). The beetle rests with the head and prothorax bent downward. When disturbed, the head, prothorax and legs are drawn closely together. It remains in this position for a few minutes, feigning death. The adults feed very little, if at all, and at summer temperatures lived for a week or more.

There are ordinarily two generations and a partial third in North Carolina (Tenhet & Bare 1951).

PROPORTIONS OF SEXES.—Heretofore, it was believed that the sex ratio of the cigarette beetle in the warehouse was about 1:1, the same as in the laboratory. Runner (1919) observed that the proportion of females seemed to be a little more than that of the males, with 36 males and 41 females emerging from four lots of laboratory cultures. He further collected 100 beetles at lights in a tobacco warehouse at Danville, Va., in July, 1911, and of these, 36 were males and 64 females. Powell (1931) noted a proportion of 46 males to 54 females from 100 individuals in the laboratory.

To determine the sex ratio of cigarette beetles collected in suction-light insect traps in the warehouse, the senior writer undertook to separate the sexes by dissection. A warehouse was selected which had never been treated with an insecticide and records were started on June 11. Beginning July 30, the same procedure was followed in a warehouse which was treated daily with an insecticide. Beetle collections were made daily. Collections of less than 100 were individually counted and dissected; for larger collections the same procedure was followed by using an aliquot of 100 beetles. The data in table 2, summarized as weekly totals, were obtained by projecting the results from the aliquot to the total number collected.

The data indicated (figure 1) that in the untreated tobacco storage warehouse the female beetles were in greater numbers for a period of 8 weeks, in June and July. Then for 3 weeks in August the pattern changed, for there were more males than females. From August 20 to the end of the collecting period, there were again more females than males. Since collections started on July 30 in the treated tobacco storage warehouse, the proportion of sexes prior to that date is unknown. Here the sex ratio appeared to be slightly different, in that more males than females were found during the period August 6 to September 3, 2 weeks longer than in the untreated warehouse. Thereafter, there were a few more females than males. It was noted that in both warehouses the males appeared to be more prevalent at the same time. The overall sex ratio in the untreated warehouse for the entire period of 19 weeks (males 476,887; females 571,027), and in the treated warehouse for 12 weeks (males 66,599; females 62,846) was approximately 1:1.

Table 1.—Life cycle of *Lasioderma serricorne* (F.) reared on flue-cured tobacco during the summer in a tobacco storage warehouse.

No. Eggs[a] Hatched	Incubation Period (Days)	No. and Sex of Adults	Life Cycle (Days)					
			Egg Stage		Larval Stage		Pupal Stage	
			Average	Range	Average	Range	Average	Range
8	4							
1	5							
2	6							
3	7	11 males	7	4 to 11	40	32 to 48	5	3 to 7
10	8	17 females	7	4 to 10	40	31 to 52	4	2 to 8
1	9							
2	10							
1	11							

[a] Deposited June 14, 1956.

FECUNDITY OF THE FEMALE.—The average number of eggs deposited, the length of adult female life and the length of the oviposition and post-oviposition periods of the cigareette beetle were determined under warehouse conditions.

In the Philippines, Jones (1913) stated that egg laying began 2 to 5 days after emergence. Powell (1931) stated that a female is rarely found that does not deposit eggs during the first 6 days of her life; furthermore, of the 100 pairs observed he noted that all females finished deposit-

Table 2.—Weekly totals of male and female cigarette beetles collected in suction-light insect traps in an untreated and an insecticide-treated tobacco storage warehouse.

Week Beginning	Number from			
	Untreated Warehouse		Treated Warehouse	
	males	females	males	females
June 11	1,672	2,648		
18	541	783		
25	140	186		
July 2	26	80		
9	693	1,107		
16	7,038	14,672		
23	31,745	59,641		
30	24,987	41,437	7,007	8,285
Aug. 6	13,936	12,261	3,448	2,953
13	16,849	14,859	6,206	5,205
20	15,440	13,450	9,392	7,668
27	95,670	111,220	22,854	22,008
Sept. 3	94,208	110,321	9,200	7,803
10	74,197	84,208	3,135	3,587
17	49,712	51,320	3,391	3,488
23	28,111	27,894	1,600	1,377
Oct. 1	15,752	17,648	288	376
8	2,407	2,769	63	79
15	3,763	4,523	15	17
Totals	476,887	571,027	66,599	62,846
Per cent of total	46	54	51	49

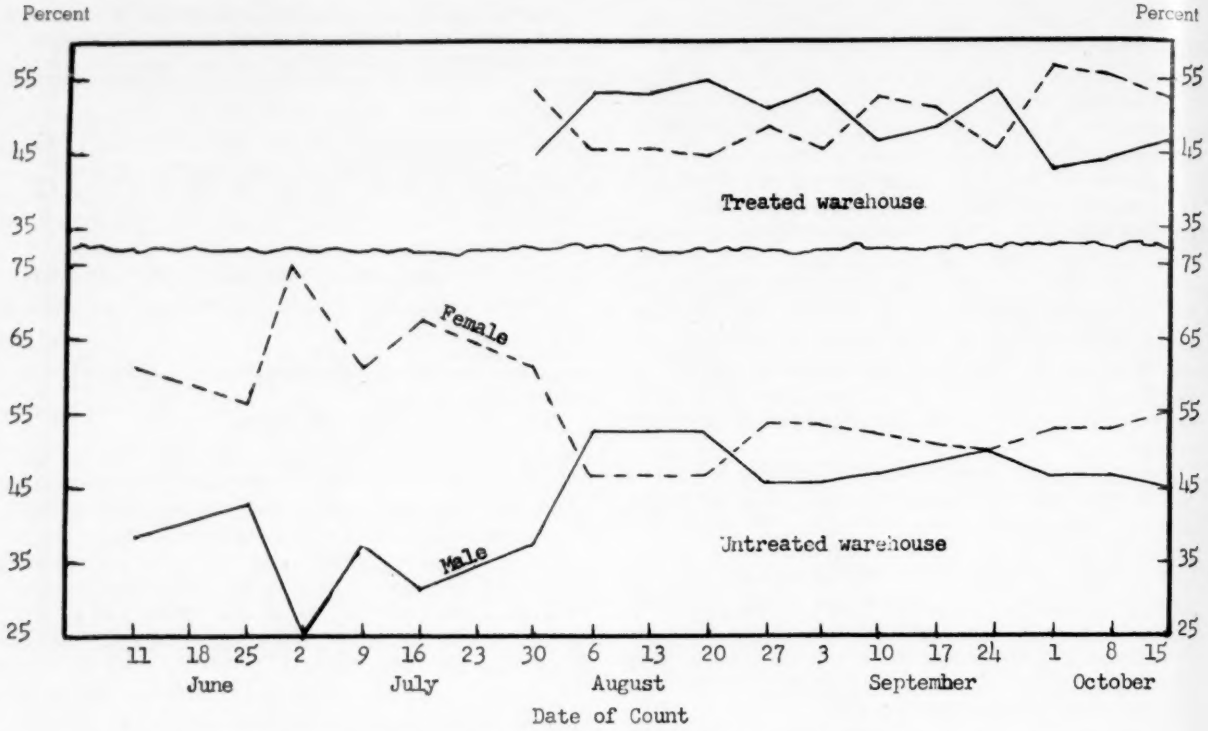

FIG. 1.—Per cent of male and female cigarette beetles trapped weekly in a suction-light insect trap in an insecticide-treated and untreated tobacco warehouse during the summer of 1956.

ing eggs within a period of 10 days after emergence. He found that the average number of eggs per female was 44. Dick (1937) observed that with the exception of one female, all oviposited and produced more eggs on the first day than on any subsequent day. He recorded the oviposition of 10 *L. serricorne* females, the food used being rolled oats with a small amount of yeast. He also noted that oviposition commenced between 6 to 8 days after emergence and continued for 6 to 9 days, during which the average number of eggs deposited per female was 76.

Jones (1913) noted that the average number of days the adult lived was 16. He did not give the sex. Runner (1919) mentioned that the adult may live 3 weeks or more.

Mossop (1932) stated that in Southern Rhodesia the adults normally live 5 to 6 weeks in the summer but longer in the winter.

In our study 105 pairs of adults were selected while in the pupal stage and caged as pairs. The pairs were placed in small glass dishes 15 mm. × 30 mm. with glass covers. A small section of split, flue-cured tobacco midrib was placed in each container for the female to use for egg deposition. The containers were kept in the warehouse where the temperature averaged 85° F. and the relative humidity was about 60%. The eggs were removed and counted daily. Each pair was kept through its entire adult life.

It was noted that the 105 cigarette beetle females deposited eggs over a period of 18 days. The average number of eggs deposited per female was 42, the minimum was 18, and the maximum was 112. Figure 2 shows that 66% of the eggs were deposited by the end of 4 days after emergence and 98% within 11 days. The average oviposition and post-oviposition period was 8 days each, the range for the oviposition period was 2 to 18 days and for the post-oviposition period 1 to 36 days. The average life of the female was found to be 18 days, the minimum 8 days, and the maximum 42 days. The life span of the male beetle was not recorded, but was observed to be slightly longer than that of the female.

GEOTROPISM—TEMPERATURE STUDY.—Frequently it has been observed that the top floors in a two- or three-story warehouse, and the top tiers of hogsheads on individual floors, are more heavily infested with cigarette beetles than the lower floors or tiers. An attempt was made to determine why the beetle flies upward in relation to gravity; whether this is an attraction to higher temperatures or the results of negative geotropism.

In a closed-type, single-floor tobacco storage warehouse, three cloth-covered, insect-tight cages were constructed. Each measured 19 feet long, 16 feet wide and 17 feet high, with the exception of Cage III which was 16 feet high. Cages I and II were constructed so the temperature remained the same as in the warehouse which housed them. These had a cheese-cloth panel, one yard wide inserted at the top and bottom of the two longer sides of the cages which were covered with heavy cotton twill cloth. Cage III was covered completely with heavy cotton twill cloth and had its ceiling 1 foot below the warehouse ceiling. Ten hogsheads of tobacco strips were placed in each cage. These hogsheads came from a warehouse which was never treated with an insecticide and were from the top (or third) tier, thus being likely to have a heavy cigarette beetle infestation. From May 14 to August 2 the

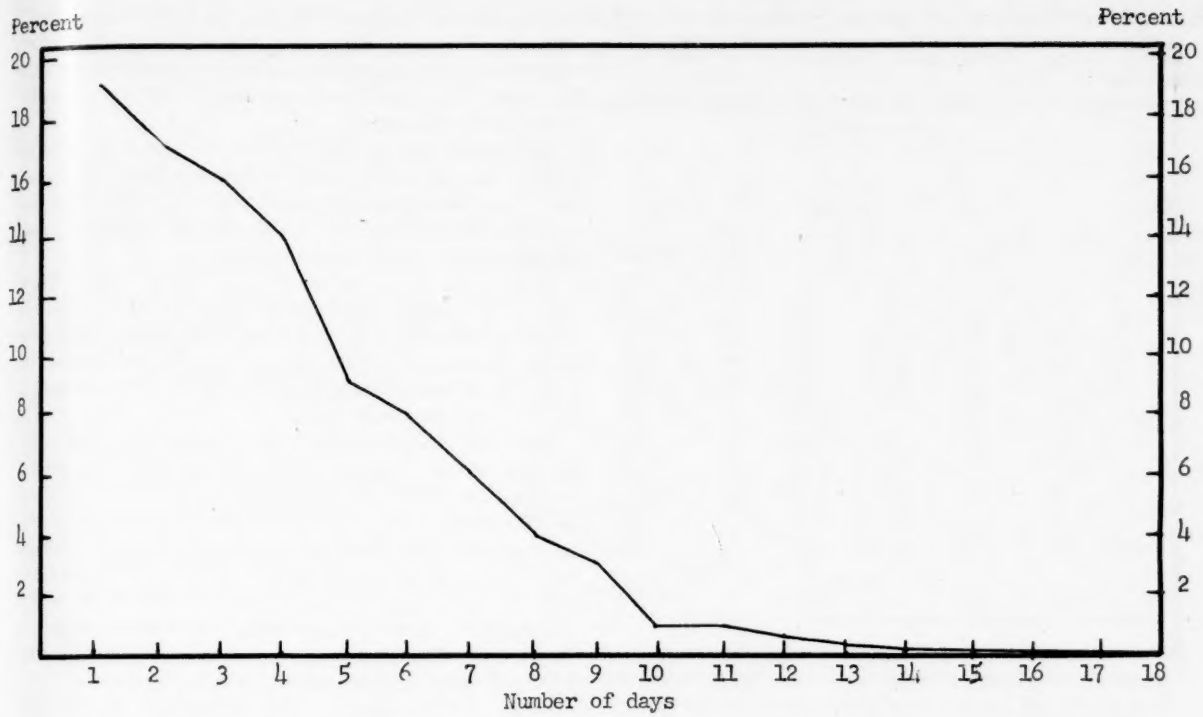

FIG. 2.—Per cent of total egg deposition from 105 cigarette beetle females recorded daily over a period of 18 days.

hogsheads remained on their sides; thereafter they were kept in an upright position. The net weight of the hogsheads ranged from 1046 to 1132 pounds, with a mean of 1101 pounds. The catch of beetles, the temperature, and the humidity were recorded daily in the three cages at the 1-foot, 6-foot and 11-foot levels above the floor.

Cage I contained six heat unit traps and six tanglefoot paper board traps. The heat unit traps were constructed of galvanized iron and were insulated except for an 8×14 inch surface on one end. On this surface a sheet of tanglefoot paper, 6×12 inches, was attached. A 25- or 40-watt electric light bulb was used inside the unit to maintain a temperature of 100 to 110° F. on the uninsulated surface. The heat unit traps were suspended on a ladder situated at the center of the two longer sides of the cage, opposite each other at heights of 1, 6 and 11 feet above the floor and about 2 feet away from the wall. The heated surfaces of the units were vertical and faced the center of the cage. The 10×16 inch tanglefoot board traps without heat, on each of which was attached a 6×12 inch sheet of tanglefoot paper, were located at the center of the shorter sides of the cage and at the same elevations from the floor as the heated traps. Instead of facing the center, these were turned toward the walls and were at a distance of $6\frac{1}{2}$ feet away from the wall.

In cage II three suction insect traps, without lights, were located in one corner facing the opposite wall and were suspended at heights of 1, 6 and 11 feet above the floor.

Cage III contained air circulation equipment. Ducts ran 7 feet out from the central circulatory fan and then extended 8 feet down to within 1 foot of the floor. The galvanized iron ducts were 8 inches in diameter, and each duct inlet was equipped with a shutter for regulation of

Table 3.—Total of weekly collections of cigarette beetles taken on or in traps in a warehouse to study effect of temperature on abundance.

	Cage I[a]						Cage II[b]			Cage III[c]			In Warehouse[d]		
	Feet above Floor						Feet above Floor			Feet above Floor			Feet above Floor		
	1	6	11	1	6	11	1	6	11	1	6	11	1	6	11
	Heated			Unheated											
	1,992	2,654	4,141	432	924	1,003	2,442	2,975	7,370	1,218	1,193	892	4,212	13,032	35,570
L.S.D. .05		1,082			162			1,293			N.S.			1,876	

[a] Unheated traps were 10×16 inch tanglefoot board traps; the heated traps were of galvanized iron.
[b] Unheated and unlighted suction traps were used.
[c] Unheated traps used and uniform temperatures were maintained by circulation of air.
[d] Unheated and unlighted suction traps in an area of the warehouse other than in cages.

Table 4.—Weekly total of cigarette beetles collected daily in suction insect traps in a tobacco storage warehouse, with weekly average of daily mean temperatures at each trap location.

Week Beginning	Feet Above Floor: 1 — Degrees F.	1 — Number Beetles	6 — Degrees F.	6 — Number Beetles	11 — Degrees F.	11 — Number Beetles
July 23	79	328	85	461	90	745
30	76	496	83	578	86	2,009
Aug. 6	79	380	86	690	90	1,590
13	80	239	86	456	90	914
20	74	129	80	358	84	1,530
27	79	401	85	1,318	89	3,400
Sept. 3	75	660	81	3,991	84	11,016
10	70	475	77	1,904	82	5,752
17	69	624	76	2,123	81	3,883
24	64	307	68	585	72	2,128
Oct. 1	69	143	73	382	78	1,823
8	57	14	66	58	69	377
15	62	16	67	128	71	403
Total		4,212		13,032		35,570

the air flow. These openings were covered with cheese cloth to prevent beetles from being drawn into the equipment. This apparatus slowly circulated the air, maintaining a uniform temperature throughout the cage. Nine board traps, to which were attached sheets of tanglefoot paper 6×12 inches, were suspended on three sides of the cage, three on each side, at heights of 1, 6 and 11 feet above the floor and near the middle of the wall.

Three suction insect traps were installed 1, 6 and 12 feet above the floor in the same warehouse which housed the three cages. These traps were suspended one above the other and were between the rows of hogsheads which were racked three tiers high. Insect collectiong began on July 23 and the same records were taken as inside of the cages.

The daily collections in the cages between June and October are shown as weekly totals in table 3. Beetle emergence from June 4 to July 9 was slight, possibly attributable to a late spring. In Cage I, the number of beetles collected on the heated traps at the three levels was about three to four times more than the number of beetles collected on the unheated traps. It was observed that the number of beetles collected on the heated traps at the 1-foot and 6-foot heights was not significantly different, but the numbers collected at the 11-foot heights were significantly greater than at the 1-foot and 6-foot heights at the 95% confidence level. The number of beetles collected on the unheated traps at the 1-foot heights was significantly lower than at the 6-foot and 11-foot heights. The latter two were not significantly different from each other at the 95% confidence level. The temperatures at the different locations were only slightly different, as the fluctuations were less than 2 degrees for any 1 day.

In Cage II nearly the same beetle catches were recorded. The count at the 1-foot and 6-foot heights showed no significant difference from each other; but at the 11-foot heights the count was significantly higher than at the 1-foot and 6-foot heights at the 95% confidence level. As in Cage I, the temperature records were only slightly different. The temperatures in Cages I and II did not exactly duplicate those existing in the warehouse outside the cages. This may have been due to the type of cloth used to cover the cages.

The beetle count in Cage III was the reverse of that in Cages I and II. In this cage the temperature was almost uniform at the various levels. Here the number of beetles collected at each level was not significantly different from the others at the 95% confidence level.

Since more beetles were collected on the heated traps and at the highest level on both heated and unheated traps in Cages I and II, and since there was no difference in the number of beetles trapped at different heights in Cage III, it would seem that the beetles were attracted to the higher temperatures.

The data from the insect traps operated in the main portion of the tobacco warehouse confirmed the above conclusions. These data, presented in table 4 and figure 3, showed that the trap catches increased progressively as the heights above the floor increased. The air temperature also increased 4 to 5 degrees F. at each higher location of these traps. As additional confirmation, when temperatures were comparatively low in cooler weather, the number of beetles trapped at the 11-foot heights was relatively much higher than at the other locations.

BEETLE EMERGENCE.—The percentage of cigarette beetles which emerge in relation to the percentage which remain in the tobacco hogsheads is unknown. To simulate conditions in hogsheads, but in a smaller more workable area, iron pipe cages were used.

A "tobacco thief" ($1\frac{10}{16}$ inches in diameter × $11\frac{1}{2}$ inches long) was used to cut cores of flue-cured tobacco from a hogshead which had passed through a thermal-vacuum unit. The tobacco, which was known to be free of insect life, was then packed into iron pipe cages at approximately the same density as in the hogsheads. These cages measured $1\frac{9}{16}$ inches in diameter by 10 inches long with a screw cap on each end. Eight cages were packed to a depth of 2 inches, 8 to a depth of 4 inches and 8 to a depth of 6 inches. The tobacco was held in place so that it could not expand, by means of a brass insert in the cylindrical cage. These inserts were metal sleeves, 4, 6 and 8 inches long, one end of which was covered with $\frac{1}{8}$ inch hardware cloth.

Experiment I.—On June 11, 1-day-old eggs, from 107 to 124 per cage, were placed on the exposed surface of the

Table 5.—Oviposition by cigarette beetles collected from within the mass of flue-cured tobacco in hogsheads.

No. of Eggs Deposited	Days in Each Period: First Eggs Deposited after Collection	Duration of Oviposition	Longevity of Female after Collection
25	2	7	12
11	3	1	5
21	3	4	13
35	3	3	19
46	3	7	11
16	4	3	9
63	4	6	25
36	5	4	27

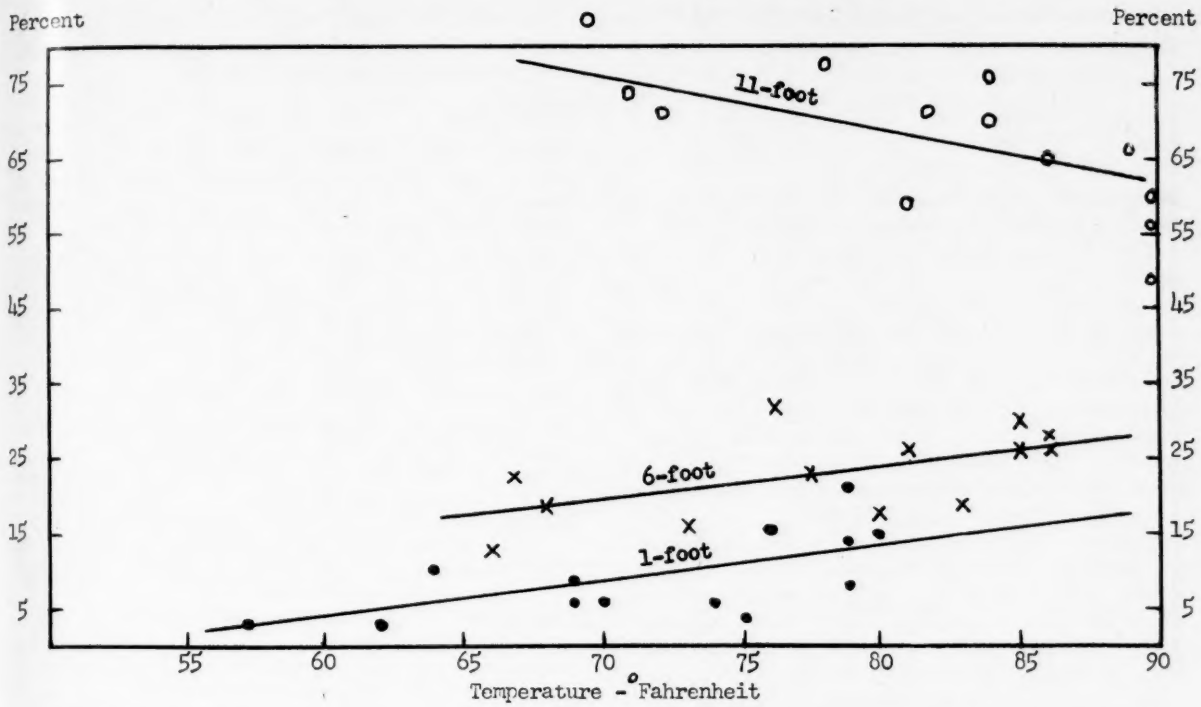

FIG. 3.—Per cent of cigarette beetles trapped over 13 weeks in a tobacco storage warehouse at 1-foot, 6-foot and 11-foot elevations above floor level during the summer of 1956.

tobacco near the cap. The moisture content of the tobacco at the time the pipe cages were packed was 11.7%, and at the completion of the experiment it was 10.9%. The pipe cages were kept in Cage I in the tobacco warehouse

After 10 weeks the pipe cages were opened and examined. The minimum depth of penetration by the beetles was $\frac{7}{16}$ inch, the maximum was $1\frac{1}{16}$ inches. The smallest percentage of eggs to hatch and reach maturity was 16%, the largest was 74%, while the average was 37%. In the pipe cages the following were found at the time of examination: 133 larvae, all living and in the 1st, 2nd, 3rd, or 4th instar; 14 living pupae, 6 females and 8 males; 873 dead adults of which 460 were females and the remainder males; and 138 living adults, 74 of which were females. All these insects were still within the mass of the tobacco.

A total of 104 eggs was found in the 24 cages. This indicated that the beetles upon reaching maturity had mated and deposited eggs without emerging from the tobacco.

Experiment II.—A similar experiment was started on August 23, but instead of placing the insect eggs at one end of the tobacco, they were placed in the center of the tabacco mass. The eggs were 1-day-old and the number varied from 110 to 128 per cage. The moisture content of the tobacco at the time the cages were packed was 13.5% and at the completion of the experiment it was 11.2%. The pipe cages were kept in Cage I.

After 9 weeks the pipe cages were opened and examined. Since the larvae penetrated the tobacco from the center of the mass where the eggs were placed, moving toward the opposite ends, the penetration was recorded in both directions. The minimum depth of penetration was $\frac{10}{16}$ inches, and the maximum was $2\frac{9}{16}$ inches. The smallest percentage of eggs to hatch was 32%, the largest was 72%, while the average was 50%. In the 24 pipe cages 1385 living larvae, either 2nd, 3rd, or 4th instar, were found. There were no eggs, pupae, or adults.

In each of the pipe cages, the beetles made circular galleries, penetrating the tobacco in all directions. There appeared to be no definite pattern of penetration. In no instance did the insects penetrate all the way through the tobacco into the air space at one end of the cage. No adults were found in the cages in Experiment II, as it was started later in the season when the temperatures were lower.

MATING OBSERVATIONS.—The cigarette beetle has been recorded as mating outside tobacco hogsheads, but there has been only speculation about its mating inside. The senior writer examined a thousand-pound hogshead of flue-cured tobacco while looking for the immature stages of the cigarette beetle and while doing this, over a period of 6 days, observed nine pair of adults *in copula*. Each pair was located about 4 to 6 inches within the tobacco from the periphery. The tobacco was in bundles or "hands."

To obtain further information on whether or not the cigarette beetle mates within tobacco hogsheads, 25 female beetles were collected from hogsheads containing tobacco strips. All were located at least 3 inches within the tobacco from the periphery. The females were placed individually in small petri dishes, along with a section of split flue-cured tobacco midrib, in a room in the warehouse. Eggs deposited were removed and counted daily.

Of the 25 females, eight deposited eggs (table 5) where-

as the remainder did not, but lived the following number of days:

♀—12 days	♂—18 days
♂—14 days	♂—19 days
♂—15 days	♀—22 days
♀—16 days	♀—27 days
♂—17 days	♀—29 days

Because only mated beetles lay eggs, it is concluded that the eight females mated within the hogshead.

Ten additional pairs of adult cigarette beetles were observed *in copula*. Four pairs were in a container of flue-cured tobacco, and the remaining pairs were noted in the collecting jar of a suction-light insect trap. These were immediately removed, placed in small petri dishes, and observations made on their mating habits. Since mating preliminaries were not observed in any of the pairs, the exact length of mating time is unknown. The periods of copulation were from 2½ to 75 minutes. The cigarette beetle has been recorded as mating more than once and this was noted repeatedly in this study.

SUMMARY.—With flue-cured tobacco as a medium it was noted that the entire life cycle of the cigarette beetle, *Lasioderma serricorne* (F.), was completed under warehouse conditions in about 52 days during the summer of 1956. The length of the incubation period was about 7 days, the larval period about 40 days and the pupal period about 5 days. The adults fed very little, if at all, and at summer temperatures lived from 1 to 6 weeks.

The sex ratio of the cigarette beetle was about 1:1 over the entire season, but there were periods during the summer when one sex was found in greater numbers than the other.

The following data were obtained from 105 pairs of adults, selected by sex when in the pupal stage, and caged as pairs: average number of eggs deposited was 42, the minimum was 18, and the maximum was 112; average life of the female adult was 18 days; average oviposition and post-oviposition period was 8 days each; 66% of the eggs were deposited within 4 days and 98% within 11 days after oviposition began.

The data obtained indicated that the beetles were probably more attracted to higher temperatures than they were influenced by negative geotropism.

In experimental pipe cages the cigarette beetle penetrated tobacco in all directions without a definite pattern. None of the beetles penetrated through the tobacco mass and emerged into the air space in the cages; thus, no information was obtained on the percentage of beetles which emerge from or which remain in the tobacco. It appeared that most of the beetles remained at or near the site where they reached maturity, mated and then deposited eggs. Mating took place both inside and outside the tobacco hogsheads.

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Ten Years of Testing Organic Insecticides as Soil Poisons against the Eastern Subterranean Termite[1]

L. A. HETRICK, *College of Agriculture, University of Florida, Gainesville*

More than 10 years ago, a series of tests was begun to determine the usefulness of chlorinated hydrocarbon insecticides as soil poisons against the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). A 5-year summary of these tests was published (Hetrick 1952). Since then, testing has been continued and a progress report is presented in this paper. The glass battery jar technique used in conduting these tests has remained the same as previously reported and it seems inadvisable to describe it again in this article. It must be remembered that this research is concerned with the incorporation of various amounts of insecticides with sandy soil and the results are not necessarily indicative of those that may be secured from chemicals tested out-of-doors in soils that are subjected to varying environmental conditions. Comparative studies, with the insecticides mixed with other soil types, have not been made.

In tables 1 and 2 results of tests are presented in order to compare the action of the insecticides tested during the first 5-year period with the results obtained during the second 5-year period. Unfortunately, at the beginning of the tests it was not known how toxic the chemicals would be to the test insect or how long they would remain effective in the sandy soil used in these tests. Dilutions were made on the basis of initial performance and show considerable variation from one chemical to another. As a

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Table 1.—Comparative toxicity of insecticides on the basis of tests conducted over a 10-year period.

Chemical	Dilution[a]	Average Days for 100% Knockdown		Number of Times Infested
		Years 1–5	Years 6–10	
DDT	1–200	3.0	2.0	36
TDE	1–200	3.3	2.6	36
	1–1,000	4.2	4.0	36
Methoxychlor	1–200	2.3	2.0	36
	1–1,000	3.0	3.8	36
BHC[b]	1–10,000	1.3	1.6	36
	1–20,000	1.3	1.0	36
	1–50,000	2.8	4.4	36
	1–100,000	3.0	4.6	36
Chlordane	1–200	1.3	1.4	35
	1–1,000	1.9	2.0	35
	1–2,000	2.0	2.4	35
	1–10,000	3.8	4.4	35
	1–20,000	4.0	4.8	35
Toxaphene	1–1,000	2.2	1.6	34
	1–2,000	2.2	2.6	34
	1–10,000	3.7	4.2	34
PCP[c]	1–200	1.4	1.8	33
SPP[d]	1–100	1.3	1.8	30
	1–500	1.6	2.0	30
	1–1,000	2.7	3.0	30

[a] All dilutions are given on the basis of one part by weight of the active insecticide in the various quantities of sandy soil indicated.
[b] Gamma isomer of BHC.
[c] Pentachlorophenol.
[d] Sodium pentachlorophenate.

word of caution, the performance of any chemical can be judged only after carefully considering the dilution; otherwise the figures may be misleading.

The initial action of DDT and its analogs (TDE and methoxychlor) was slow and numerous dilutions were not made with these chemicals for this reason. With the exception of one dilution (methoxychlor 1–1,000), this group of chemicals exhibited increased speed of action against the test insects in the period of 10 years. However, it must be remembered that a dilution of 1–200 represents approximately 0.5 pound of active insecticide in a cubic foot of sandy soil. Likewise, a dilution of 1–1,000 represents approximately 0.1 pound of insecticide in a cubic foot of sandy soil. Such heavy application would not be justified in the light of other available chemicals that are effective in much smaller amounts.

The action of BHC (gamma isomer) has been outstanding. Dilutions of 1–10,000 and 1–20,000 have remained consistently rapid in their toxic action to the test insect. At the extreme dilutions of 1–50,000 and 1–100,000, kill of the test species was not as rapid during the second 5-year period as during the first 5 years of testing. The extreme dilutions represent the minimal amounts of only 0.002 pound (0.9072 gram) and 0.001 pound (0.4536 gram) of insecticide per cubic foot of sandy soil.

Chlordane exhibited a consistent but slight slowing of action during the second 5-year period. The dilutions of 1–10,000 and 1–20,000 were far behind comparable dilutions of BHC. Although the speed of action of the various chemicals may be of little importance in actual control

Table 2.—Comparative toxicity of insecticides on the basis of tests conducted over a 7-year period.

Chemical	Dilution[a]	Average Days for 100% Knockdown		Number of Times Infested
		Years 1–2	Years 3–7	
Aldrin	1–5,000	1.1	1.0	12
	1–10,000	1.0	1.4	12
	1–25,000	1.6	1.4	12
	1–50,000	1.4	2.2	12
	1–100,000	1.4	2.2	12
Dieldrin	1–5,000	1.0	1.0	12
	1–10,000	1.0	1.0	12
	1–25,000	1.1	1.0	12
	1–50,000	1.0	1.2	12
	1–100,000	1.1	1.2	12
Heptachlor	1–5,000	1.1	1.4	11
	1–10,000	1.1	2.4	11
	1–25,000	1.3	3.2	11
	1–50,000	1.7	2.4	11
	1–100,000	1.7	3.2	11

[a] All dilutions are given on the basis of one part by weight of the active insecticide in the various quantities of sandy soil indicated.

projects, it does have some value for indicating relative toxicities in testing work.

With the exception of the 1–1,000 dilution, toxaphene has shown decreasing speed of kill. It should be remembered that a toxaphene dilution of 1–20,000 failed early in the course of the tests (5 months).

Pentachlorophenol and its sodium salt, sodium pentachlorophenate, have been widely publicized as termite soil poisons. These chemicals are satisfactory if the dosage is adequate. However, it should be remembered that the more dilute dosages of these chemicals (not shown in table 1) became nontoxic to the test insect during the early months of testing.

Although tests with aldrin, dieldrin, and heptachlor have been in progress only 7 years, indications are that they will retain their toxicity in sandy soil to subterranean termites, far beyond this time. Minimal amounts of these chemicals are needed and their superiority to other chemicals is obvious because of the small amounts required. Only BHC, in the tests for 10 years, approaches the performance of these chemicals in such small amounts.

SUMMARY.—Minimal amounts of gamma isomer of BHC have remained toxic to the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), for more than 10 years in sandy soil under the conditions of the tests described herein. Under the same conditions, aldrin, dieldrin and heptachlor have remained toxic in minimal amounts for 7 years of testing. Although chlordane, DDT, TDE, methoxychlor, toxaphene, pentachlorophenol, and sodium pentachlorophenate have remained toxic over the 10-year period, larger amounts of these chemicals were required in the treated soil used in these tests.

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White Clover Pollination with Low Honey Bee Population[1]

H. B. GREEN, *Mississippi Agricultural Experiment Station, State College*

White clover seed producers in Mississippi usually have not harvested large acreages on a single farm, and seed production has usually been a by-product of clover planted for grazing. Due to the large expansion of grassland farming there are many acres of plants flowering during the blooming season of white clover. This fact has caused concern to the seed producers, since it is known that the crop must be cross-pollinated, as it has a very high degree of pollen self-incompatibility. The peak of the blooming cycle usually occurs about the early part of May, before native bees become plentiful. Therefore, honey bees must be depended on largely for pollination of a full seed crop.

Vansell (1951) reported that Ladino clover (which is a selection of white clover) has a very low nectar production. White clover, however, is probably one of the most important sources of nectar for honey bees in Mississippi considering all sections of the state; but there are several other legumes which may compete. Honey bees will work vetches and crimson clover, as well as white clover when all are blooming at the same time.

Obervations and counts in white clover fields in various parts of the state during blooming time in 1952, 1953, and 1954 seemed to indicate that very few honey bees were found in some locations, yet seed yields from these fields were very good. Cage tests have indicated that an average of slightly more than one honey bee per 100 heads of blooming clover resulted in as good seed set as was found with more than four bees per 100 blooms (Green 1956). It was felt that more accurate field data were needed before recommendations could be made; therefore a test was conducted in 1955 on a large cattle farm near Port Gibson, Mississippi, where the seed crop on 400 acres of white clover was harvested.

METHODS.—It was hoped that several of the fields on this large farm could be used in the test to serve as replications, but for various reasons all but one field was unsuitable. The one selected was uniformly tilled, fertilized and seeded the previous year and had an excellent stand of clover. It was located on a second bottom of Bayou Pierre. The first bottom or flood plain had meadows of white clover on it, but about half was sound hardwood timber. The test field was about 600 yards wide and extended about 2000 yards away from the bayou flood plain to the first hills. The three sides of the test field away from the bayou were bounded by other large fields of clover.

There were three average colonies of Caucasian bees on one side of the test field and four on the other. Each of these bee yards was located in the adjacent clover fields about 1000 yards from either side of the test field. Before the test was started, careful observations were made of the bees working in the field. An average of about 7% of the bees were yellow Italian bees. It was thought from coursing bees from the field at various points that about six or eight other colonies were probably in bee trees off the test area. Therefore it seemed probable that not more than 15 colonies of bees were available on the several thousand acres of the farm to pollinate the 400 acres of clover for seed.

When the test was begun in mid-April, six very strong colonies of Italian bees were placed in the test field near the end that was nearest the hills. This raised the estimated honey bees available to one colony for each 15 to 20 acres, which was still a very low population considering what has been regarded necessary for good pollination of white clover. After the Italian bees were added, the percentage of Italian bees working the clover rose from about 7 to 50%. Very few Caucasians were seen within 100 yards of the Italian colonies, however. Very few wild native bees were seen on the clover during the test period.

The six colonies of bees which were added were placed across the field at intervals of 70 yards, which is roughly the dimension of one side of a square acre. Stakes 5 feet tall were placed at intervals in front of the six hives to designate areas for the making of counts and seed collections. The first stakes were 35 yards from the hives, the second row was 70 yards from the first, and the third 70 yards from the second. The fourth through the eleventh rows of stakes were placed at 100-yard intervals. The last stakes were 975 yards, or almost 0.6 mile, from the bee hives.

It is realized, of course, that the six parallel areas where counts were made cannot be considered true, unbiased, randomized replications of plots. Also it is known that soil variations existed, and that the test crossed them. For these reasons the counts made were more of the nature of subsamples, but they were considered as replications in the analysis of the data collected. It is known that the number of blooms per square yard and the number of pods per head and seeds per pod are influenced by the plant vigor (Green 1956). Therefore the consideration of such data in the analysis also supports the use of the rows of areas as described above as replications.

The average bloom count per square yard, for 2 square yards at each stake, was made each week for 6 weeks starting on April 27. At the same times the number of bees per 100 blooms were counted on 1000 blooms at each stake. The counts were made during mid-day when bee activity was fairly constant.

Fifty ripe heads were picked at each stake on two occasions. The first picking was made during the third week of May, as the early blooms became ripe. The second picking was made during the first week of June when the "peak of bloom" crop became ripe, just prior to the application of a desiccant before the field was harvested. In the laboratory the heads of each sample were shattered by hand and the pods were counted. The pods were broken by rubbing them through a screen sieve, and the seeds were carefully cleaned by hand and counted. From these data the seeds per pod were calculated for each sample.

RESULTS.—The data in table 1 indicate that as the

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Table 1.—Clover blooms, seed and honey bee counts in relation to distance from hive.[a]

Yards from Nearest Hive	Blooms per Square Yard			Bees per 100 Blooms			Seeds per Pod		Pods per Head at Peak
	Early	Peak	Average	Early	Peak	Average	Early	Peak	
35	25.5	83.5	52.5	1.03	0.48	0.63	3.42	3.10	52.5
105	25.5	82.5	56.5	.90	.63	.63	3.38	3.00	52.7
175	26.2	88.2	57.2	.90	.53	.67	3.37	2.90	55.0
275	16.7	73.3	49.2	.80	.43	.44	3.11	2.83	48.8
375	21.0	71.8	44.5	.57	.48	.42	3.04	2.89	52.2
475	24.0	76.7	47.5	.58	.48	.45	3.28	2.70	51.0
575	14.7	74.5	47.0	.75	.50	.54	3.00	2.80	50.3
675	20.3	72.2	46.8	.83	.53	.52	3.03	2.63	52.2
775	18.2	70.8	45.5	.65	.43	.50	2.88	2.78	50.7
875	13.0	65.8	42.7	.72	.51	.46	2.95	2.57	48.8
975	16.7	41.8	41.8	1.05	.26	.52	2.93	2.59	49.8
Average	20.2	72.8	48.3	.79	.47	.52	3.12	2.79	51.3
L.S.D. at 5% level	8.8	23.7	8.2	ns	ns	.13	.22	.30	3.01
at 1% level	—	—	11.0	—	—	.17	.29	—	4.02

[a] Early count made April 27; peak of blooming season May 18; averages are for six counts.

blooms per square yard increased from April 27 to May 18, the bees per 100 blooms decreased. A row of averages for the columns is included in the table, and it may be seen that although the actual number of bees per square yard increased over 100% on May 18, the number per 100 blooms was only about 50% as great, due to the increase of blooms per square yard.

It was stated earlier that it has been found that slightly more than one honey bee per 100 blooms gave efficient pollination, but in this test that level was approached only in the early season count. In the second count the seeds per pod showed a decrease over the early bloom, but it was not proportional to the decrease in bees per 100 blooms. It has been found that such a decrease occurs even when bees are plentiful (Green 1956). It has also been found that an average production of 2.79 seeds per pod at "peak of bloom" may be considered an excellent yield.

The sampling technique for bees per 100 bloom counts was not adequate to show differences at any single count, although 1000 blooms were examined on each area sampled; however, an average of the six counts for the season showed significant differences. The activity near the bee hives was greater than in some other areas, but some of the more distant counts were not different from those near the hives. These higher counts at the greater distances were not nearer the Caucasian hives mentioned, and were not in a pattern which might suggest their proximity to bee colonies. Other studies have shown that honey bees may work white clover at the rate of four or more per 100 blooms, or a population requiring at least one strong colony per acre. No such populations were encountered in this study and it may be noted that although the differences in bees per 100 blooms for the season were significant, there were actually no great differences among them. This indicates that the bees dispersed over a wide area from the hives, although they flew over good bee pasture in so doing. Further evidence of this dispersal was noted while the bee and bloom counts were being made. In the open field, the bees used the stakes designating the areas for making of counts as references in flying to their hives, and on several occasions it was necessary to repeat some bee counts, because the returning bees massed in such numbers near the observer that foraging bees in the area were frightened away. When the observer moved a few yards to the side, the bees continued their journey. This condition was noted as far away as 500 yards from the Italian colonies.

It was noticed during the time that the counts were made that more bees were usually found working clover where the bloom was more plentiful. This suggested an explanation for the variations found in the bee counts. Although the field had been uniformly treated by the operator, there was a history of several uses of the land, and there were some variations in subsoil drainage and other soil characteristics. The first three count areas in each row were on land which had been cropped in rows and was a more uniformly fertile portion. The remainder of the field had been in timber with clearings used for pasture, and it was not as uniformly fertile.

To study the interrelated aspects of the problem better, simple correlations were determined for seeds per pod at "peak of bloom" with: (1) blooms per square yard (average for the season); (2) pods per head (average for the season); (3) bees per 100 blooms (average for the season); (4) (average blooms per square yard) (average bees per 100 blooms). Tests of significance showed that there was a highly significant correlation (.394) for seeds per pod and pods per head. There was also a significant correlation (.598) for blooms per square yard and bees per 100 blooms. There was no significant correlation for seeds per pod and bees per 100 blooms.[2] Stated more simply, heads with more pods had more seeds per pod, and areas with greatest numbers of blooms had more bee activity per 100 blooms, but more bee activity did not necessarily result in more seeds per pod.

CONCLUSIONS.—It may be concluded therefore that although honey bees are necessary for cross-pollination, conditions other than the number of pollinators limit seed production beyond a certain point. The data here reported indicate that the limit of seed production of the

[2] The author wishes to acknowledge the assistance of Dr. Walter J. Drapala, Statistitian, Mississippi Agricultural Experiment Station with this correlation problem

plants was reached with a very low population of bees. When calculated from the averages in table 1, there were only an average of 0.26 bee per square yard for the season, or 0.34 for the "peak of bloom." Therefore there were about 1258 bees per acre for the season's average, or 1646 for "peak of bloom." It has been estimated that there are about 20,000 field bees in a strong colony; therefore the 20 colonies on the 400 acres in the area of the test could not supply more than 1000 bees per acre. This would seem to indicate then, that between 258 and 646 bees per acre flew to the field from more than a mile away; however, it is also possible that there were more colonies of bees in trees in the area than were estimated. Regardless of the source of the bees, it might be liberally considered that there were not more than 40 colonies working on the 400 acres, or 10 acres per colony.

This represents a wide departure from the one or more colonies per acre usually recommended for good pollination; but since the known number of colonies of bees and the observed number of bees seem to agree fairly closely, and the seed yield was somewhat above average, it seems logical to assume that no more honey bees were needed.

The reason white clover may produce good seed crops with few bees may perhaps be explained by the fact that each floret on the head produces a pod which may have six or seven seeds if it is cross-pollinated and if the plant's vigor can support the seed production.

SUMMARY.—A test of white clover pollination was conducted on a field about 600 yards wide and 2000 yards long in an area where the known and estimated number of honey bee colonies averaged 15 to 20 acres per colony. Bee and bloom counts and data concerning pod and seed production were recorded and simple correlations were calculated. It was found that more bloom attracted more bees, and larger heads had more pods, but more bee activity did not increase seed production.

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Resistance to the Spotted Alfalfa Aphid in Lahontan Alfalfa[1]

W. L. HOWE, *Entomology Research Branch*, and OLIVER F. SMITH, *Field Crops Research Branch, Agric. Res. Serv., U.S.D.A.*

The spotted alfalfa aphid, *Pterocallidium* sp., during its rapid spread throughout the southwestern United States in 1954 and 1955, probably infested every alfalfa nursery in its path. Observations made by entomologists, agronomists, and others showed that Lahontan alfalfa survived and continued to make good growth under infestations which severely injured or killed other varieties (fig. 1). Examination of Lahontan plants showed a low population of aphids and only a small amount of the characteristic sticky honeydew, whereas high populations of aphids and abundant honeydew were present on plants of other varieties. Resistance in Lahontan was also evident in seedling stands. Very few seedlings of Lahontan were killed by the aphids, while mortality approached 100% in most other varieties.

Lahontan was bred by Smith (1955) primarily to fill the need for a winter-hardy variety resistant to the stem nematode and bacterial wilt. It was released co-operatively by the U. S. Department of Agriculture and the California and Nevada Agricultural Experiment Stations for limited plantings in 1954, almost simultaneously with the introduction and spread of the spotted alfalfa aphid in the Southwestern States. Since the aphid now occurs over a broad climatic range, Lahontan alfalfa will not meet the requirements of an aphid-resistant variety for all areas where this aphid occurs. Consequently, breeding programs have been initiated by the U. S. Department of Agriculture and State agricultural experiment stations to transfer the aphid resistance of Lahontan to varieties adapted for regions where Lahontan is not suitable.

Plant breeders need information on the nature and degree of resistance in Lahontan, its parent clones, and various progenies produced in their breeding programs. It is necessary, therefore, to determine levels of resistance in individual plants and in plant populations. Fortunately, techniques that previously had been utilized in studies of pea aphid resistance in alfalfa proved adaptable for use in studies of resistance to the spotted alfalfa aphid. The level of resistance in Lahontan was usually determined by a comparison with three other varieties—African, Caliverde, and California Common 49. The work reported herein was conducted in 1955 and 1956, largely at the U. S. Department of Agriculture alfalfa nursery in Orange County, California.

METHODS.—The rapid reproductive and developmental rate of the spotted alfalfa aphid made it possible to identify resistant plants in a short time by various infestation techniques. Populations initiated by three females caused severe injury to susceptible plants in 14 to 16 days when day temperature reached 95° F. Under identical conditions 100% mortality of introduced aphids occurred within 72 hours on highly resistant plants. In addition to the level of resistance, it was also possible to determine the effect of plant resistance on the biology of the aphid.

Two types of tests were used to evaluate resistance. One considered the ability of the aphids to live and to reproduce on individual plants. This type of test is a measure of antibiosis, or the adverse effect of the plant on the biology of the insect. It had been used previously by Dahms & Painter (1940) and by Jones *et al.* (1950) in studies of pea aphid resistance in alfalfa. The other considered only the injury to the plant caused by the aphid. Evaluations of resistance in seedlings were conducted in

[1] Accepted for publication January 3, 1957. Cooperative investigations between Entomology Research and Field Crops Research Branches, and the California and Nevada Agricultural Experiment Stations. The Irvine Company, Tustin, Calif., provided some of the facilities for conducting these investigations. J. W. Scrivener, Entomology Research Branch, assisted in carrying out the resistance tests.

FIG. 1.—Growth and stand survival of Lahontan alfalfa (left foreground) after severe spotted alfalfa aphid attack nearly destroyed the two susceptible varieties on each side, at Yuma, Ariz. (Courtesy of Arizona Agricultural Experiment Station)

the greenhouse and those in mature plants were carried out in the nursery. In all tests only vigorously growing plants were used.

RESISTANCE IN SEEDLINGS.—Greenhouse tests were conducted during the winter of 1955 to determine the degree of resistance in seedlings from random populations of Lahontan, African, and Caliverde alfalfas. Studies were made of aphid-fecundity rates on individual plants and of plant responses to feeding by large populations of aphids. Single nymphs, in the third or fourth instar, were placed on the plants and allowed to grow to maturity and reproduce. Their offspring were counted and removed daily over a 21-day period.

The results are given in table 1. On plants rated as highly resistant there was no reproduction of aphids. On plants rated as resistant, the reproduction was not more than one-third of that on Caliverde; on those rated as intermediate in resistance it was one to two-thirds as great, and on those rated as susceptible at least two-thirds that on Caliverde.

There was wide variation in the reproductive rates on individual Lahontan seedlings, suggesting that considerable segregation for antibiosis occurs within the variety. This is attributed to the differences in resistance among the five parental clones which constitute the variety.

Table 1.—Aphid reproduction on seedling plants of three varieties of alfalfa grown in the greenhouse and classified as to resistance on the basis of aphid reproduction.

Variety	Number of Plants Infested	Number of Nymphs Produced per Day	Resistance Index[a]	Number of Plants in Each Class			
				Highly Resistant	Resistant	Intermediate	Susceptible
Lahontan	23	1.2	5	5	2	6	10
African	12	2.1	9	0	1	2	9
Caliverde	12	2.4	10	0	0	0	12

[a] 10 = Maximum susceptibility.

Symptoms of aphid injury were much more evident on African and Caliverde than on Lahontan, when the three varieties were fed on by approximately equal numbers of aphids. Most plants of Caliverde and African developed a characteristic yellowing along the veins of the leaflets as a result of feeding by a single adult and a few newly born nymphs. This type of injury was also observed on some of the Lahontan seedlings after they had been fed on by the aphids. The spread of the injury into several leaflets suggests that a toxin which becomes systemic may have been injected into the plant. The apparent ability of Lahontan to tolerate such a toxin is believed to contribute materially to its high resistance. The highly resistant plants of Lahontan developed only an oval chlorotic spot on the infested leaflet after sustained attack by large numbers of aphids. When the leaflet was completely yellow, it usually dropped off.

Seedling plants of Lahontan showed a high resistance to the aphid. Several hundred seeds of Lahontan, African, and Caliverde were broadcast in divided flats and the seedlings were infested with aphids soon after the plants emerged. Two weeks later the aphids had killed all the plants of Caliverde but none of Lahontan. Continued infestation in Lahontan resulted in moderate seedling mortality. Some seedlings of African showed tolerance to the aphids, but nearly all were killed or severely injured.

RESISTANCE IN MATURE PLANTS.—Mature plants of susceptible alfalfa varieties are more capable of surviving severe aphid infestations than seedlings. Aphid resistance in seedlings is important during the short period of stand establishment, but mature plants are subject to attack at intervals throughout the life of the stand. Since periodic heavy infestations cause severe losses in forage production and a gradual reduction in stand, a practical evaluation of resistance includes a measure of growth capacity under conditions favorable to a rapid increase in aphid populations.

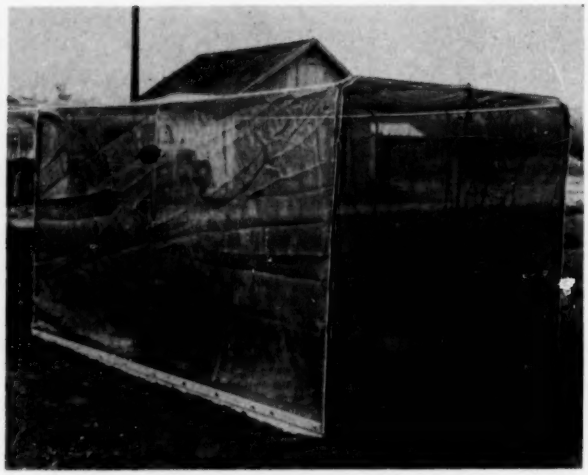

FIG. 2.—Cage placed over alfalfa plots for determining plant injury by spotted alfalfa aphid.

Observations on the performance of Lahontan in comparison with other varieties were made during the spring of 1956 in field plots at the Southern Nevada Experiment Station at Logandale. Heavy populations injured and drastically reduced the first crop of 3-year-old stands of African, Caliverde, and California Common 49. Under the same conditions Lahontan produced excellent green forage which was over 2 feet high in the prebloom stage. Honeydew was evident on Lahontan, but not in sufficient quantity to be economically important.

The first crop of hay was harvested from these plots on May 2. The dry-weight yields, in pounds per plot, were as follows: Lahontan 13.3, African 4.6, Caliverde 3.3, and California Common 3.7. When the spotted alfalfa aphid was not a factor in forage production, African alfalfa produced at least as much forage on the first crop as Lahontan. While some of the high yield of Lahontan is attributable to its good stand maintenance under heavy aphid infestations, the 289% increase over African, when both varieties are subjected to heavy aphid infestation, illustrates the value of a resistant variety over one that is not resistant.

During the summer of 1956 the growth and plant mortality of Lahontan, Caliverde, and various crosses and polycross progenies of selected plants were determined when grown in rows under Lumite cages and exposed to aphid infestations. The purpose of the cages was to exclude primary predators, particularly the convergent lady beetle, *Hippodamia convergens* G.-M. Each cage consisted of a tubular metal frame 6×10×5 feet, covered with 20-mesh plastic screen with a zipper at one corner to permit entry (fig. 2). The bottom edge of the screen was held securely between the galvanized metal base and outer metal strips by tightening with wing nuts.

In March 1956 different lines were seeded in 4-foot rows 6 inches apart in plots 4×9 feet. A cage was placed over each plot on July 11. The alfalfa inside was cut and removed, and the stubble sprayed with TEPP emulsion to kill predators. On July 13 approximately 5000 cage-reared aphids were introduced into each cage by shaking heavily infested stems over the entire plot. Aphid population increased on the new growth, but injury to susceptible varieties in most plots was not severe enough to obtain a critical test. This difficulty was overcome by cutting back each row on July 22 and leaving the clipped stems between the rows. Aphids migrated from the clipped stems to the stubble, and the new crown growth was exposed to a heavy infestation of aphids.

Table 2.—Reactions of alfalfa plants after exposure to severe infestation of the spotted alfalfa aphid.

VARIETY OR LINE	GROWTH OF PLANTS	PER CENT MORTALITY OF PLANTS
Lahontan	Excellent, tall	0
Caliverde	None, all foliage killed	97
Ranger 533	None, all foliage killed	72
Ranger 535	Fair, severely injured	3
C-84×Ranger 5	Excellent, tall	4
Nevada 13-11	Good, short with heavy honeydew	4

Observations were made on plant growth and injury on August 3, and on plant mortality on September 7. Table 2 records the wide differences that appeared among the varieties and lines in the test. Lahontan made excellent tall growth and none of the plants were injured. Caliverde made poor growth and most of the plants were eventually killed. Other progenies showed different degrees of injury.

For more detailed studies on resistance, cages of the type shown in figure 3 were used to confine aphids to single stems of alfalfa and exclude predators. Each cage consisted of a reinforced 14-mesh copper-screen cylinder, 5 inches in diameter and 11 inches high, covered on the outside and upper end with 26×32 mesh orlon marquisette. The cage base was 6×6 inches and constructed from $\frac{3}{8}$-inch all-weather plywood, with a $\frac{3}{4}$-inch center hole. The base was attached with a copper wire to a 4-inch ringstand support that could be easily adjusted to the height desired on a $\frac{3}{8}$-inch galvanized rod driven into the ground adjacent to the plant. Plastic foam $\frac{1}{2}$-inch thick, glued to the upper surface of the base, provided a close fit between the lower edge of the cage and the base. The cage was hooked securely to the base by two detachable springs. The assembly was set over an alfalfa plant in such a way that a single stem protruded up through the hole in the base. Only 2 or 3 inches of the stem were caged. The cage was raised as the plant grew taller, or the stem was clipped if the terminal reached the top. The shading of the stem by the cage was probably little greater than that on the lower leaves in a normally dense stand of plants.

Aphid-reproduction tests were carried out on 1-year-old plants selected at random from the nursery varietal

Table 3.—Class distribution of random populations of of plants from four varieties of alfalfa based on spotted alfalfa aphid reproduction on individually caged stems.

VARIETY	HIGHLY RESISTANT	RESISTANT	INTERMEDIATE	SUSCEPTIBLE
California Common	0	0	0	12
Caliverde	0	0	0	12
African	1	3	4	16
Lahontan	6	4	7	3

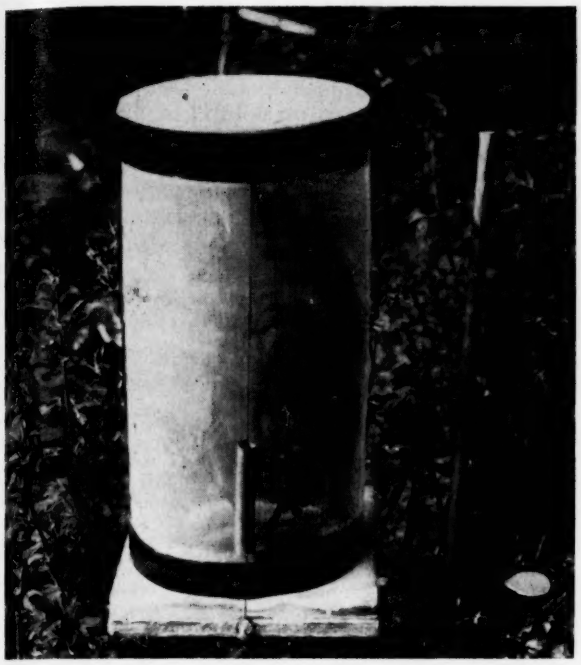

FIG. 3.—Wire-screen cage covered with fine-mesh orlon used to study resistance of spotted alfalfa aphid in single plants of alfalfa.

planting. Each stem isolated in a cage was infested with three third-instar nymphs, and observations were made every 3 or 4 days. Missing or dead nymphs were replaced until reproduction was observed, and then populations were allowed to increase with as little disturbance as possible. The number of leaflets infested and the degree of infestation at the end of 20 days were used as measures of resistance in individual plants. Plants were classed as highly resistant if no reproduction or survival was observed; resistant if only light scattered populations were produced, with no congregated colonies on individual leaflets; intermediate in resistance if 1 to 8 leaflets harbored colonies of 10 or more aphids; and susceptible if 9 or more leaflets were so infested.

Results are given in table 3. Heavy infestations developed on all California Common and Caliverde plants, and the varieties were classed as susceptible. Only a light infestation developed on Lahontan, showing that the variety has a high degree of resistance. A fairly heavy infestation developed on African, showing that it has only a low level of resistance. Repeated introduction of healthy nymphs to resistant plants from either Lahontan or African was always followed by a low survival of nymphs.

Specific comparisons of the level of antibiosis in seedlings and mature plants were not made. However, antibiosis appears to be more pronounced in mature plants of African and Lahontan than in greenhouse-grown seedlings of these varieties.

Certain aphid-behavior patterns became evident during the course of this work and in various selection tests not reported. Nymphs born on leaves of resistant plants were restless and tended to scatter over the leaflets, while those born on susceptible plants often remained congregated near the mother. Nymphs placed on resistant leaflets frequently preferred to feed on the midrib, petiole, or stem rather than on the greener leaf tissues. They also changed feeding sites more often than those on susceptible foliage. Highly resistant nursery plants were easily identified by introducing a dozen or more aphids to individually caged stems and merely observing subsequent aphid build-up and plant injury without detailed counts. Under summer conditions no aphids survived or only light scattered populations developed on resistant plants, while large numbers survived on the susceptible plants and the plants were usually killed in 2 to 3 weeks.

RESISTANCE IN LAHONTAN PARENTS.—The level of resistance in each of the five parental clones of Lahontan was determined by observing aphid survival and reproduction on individually caged stems during July and August. The plants were growing out of doors in fertile soil.

Test 1 compared the reproductive rates of adult wingless females over a 21-day period. Since data were desired only from new adults, third-instar nymphs were introduced a number of times on several stems in order to obtain a single reproducing female. Data on their survival and reproductive rates are given in table 4. The introduced aphids were dead within 48 hours after being placed on clones C-84, C-89, and C-900. Reproduction occurred on C-901 and C-902, but at a slower rate than on the susceptible check.

In test 2 the survival of newly born first-instar nymphs was determined. These nymphs, born from several reproducing adults previously placed on the plant, failed to survive on C-89 and C-84. Some survived on the other clones (table 4).

Table 4.—Reaction of the spotted alfalfa aphid on nursery-grown parental clones of Lahontan alfalfa.

Parental Clone	Field Test 1			Field Test 2		Laboratory Test		
	Number of Third Instars Introduced	Number of Nymphs Maturing	Reproductive Rate (Nymphs per Day)	Number of First Instars Introduced	Per Cent Survival	Per Cent Survival of First or Second Instars After (hours)		
						24	48	72
C-84	15	0	—	13	0	5	0	—
C-89	10	0	—	14	0	0	—	—
C-900	7	0	—	13	15.4	0	—	—
C-901	6	3	2.7	14	42.9	90	80	80
C-902	7	5	2.3	21	100.0	75	65	55
California Common (check)	6	3	3.9	43	100.0	95	95	90

In another test stems from vigorously growing nursery plants were clipped of all foliage except a single well-developed trifoliate, and the trifoliate was tested for resistance to the aphid. On each plant one trifoliate from each of four stems was tested. Each stem, after being cut from the plant, was inserted in a vial of distilled water and the vial was plugged with cotton to hold the stem in an upright position. The vials were then placed on a laboratory bench and each trifoliate was infested with five nymphs. Aphid survival was noted after 24 and 72 hours. The results were consistent with those obtained in the tests on plants growing in the field.

In these studies three of the parental clones of Lahontan—C-84, C-89, and C-900—showed a high level of resistance to the spotted alfalfa aphid; clones C-84 and C-89 were practically immune. Clones C-901 and C-902 were classed as intermediate in resistance, C-902 being slightly more resistant than C-901. These results were not always duplicated, however, for in later tests at cool fall temperatures nymphs introduced on the resistant clones matured to adults that reproduced at low rates. Also under field conditions reproducing forms of the aphid, probably migrants, were found on the resistant plants. As in pea aphid resistance studies reported by Dahms & Painter (1940), optimum temperatures for reproduction may be lower on resistant than on susceptible plants. Under severe field infestations in Nevada and California the polycross progenies of these five plants have, in general, reflected about the same level of resistance as shown by the plants themselves.

SUMMARY.—The spotted alfalfa aphid, *Pterocallidium* sp., caused severe injury to alfalfa during its spread over southwestern United States in 1954–1955. However, Lahontan alfalfa made good growth under infestations which severely injured or killed plants of other varieties. Lahontan plants showed a low population of aphids and only a small amount of the characteristic sticky honeydew, whereas high populations of aphids and abundant honeydew were present on plants of other varieties.

Lahontan had a high level of resistance in both the seedling and mature plant stages of growth. Antibiosis and tolerance appear to be the most important mechanisms of resistance. Within 2 weeks after seedlings of Caliverde, African, and Lahontan were infested with aphids, all the seedlings of Caliverde were killed, but none of the Lahontan seedlings. Seedlings of African alfalfa were tolerant to the aphid, but nearly all were killed or severely injured. When older plants of these three varieties and polycross progenies of selected plants were tested under plastic cages to exclude predators and allow the aphids to increase rapidly, Lahontan made excellent tall growth and no plants were killed, whereas the other varieties were killed or the top growth was injured severely.

Individual Lahonton seedlings and mature plants that supported aphid reproduction and growth were far more tolerant to injury than plants of Caliverde or California Common.

The value of an aphid-resistant variety was shown by field-plot tests in southern Nevada. In 1956 heavy populations of aphids injured and drastically reduced first-crop hay of 3-year-old stands of African, Caliverde, and California Common 49, but caused no injury to Lahontan, which produced excellent green forage over 2 feet in height. On the basis of dry weight per plot for first crop, Lahontan produced 289% of African, 359% of California Common 49, and 403% of Caliverde.

The level of resistance in Lahontan was determined by observing aphid survival and reproduction on leaves of individually caged plant stems. The aphids placed on plants C-84, C-89, and C-900 died within 48 hours. Aphids placed on plants C-901 and C-902 reproduced but at a slower rate. C-84, C-89, and C-900 were therefore classed as highly resistant, and C-901 and C-902 as intermediate in resistance. C-901 is the least resistant plant.

Injury to alfalfa by the spotted alfalfa aphid may be due to a toxin that becomes systemic when injected into the plants. The apparent ability of Lahontan to tolerate the toxin is believed to contribute materially to its high resistance.

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Experiments with Sprays, Dust, and Aerosols for the Home Garden[1]

L. P. DITMAN, H. B. OWENS, and FLOYD P. HARRISON, *Department of Entomology, University of Maryland, College Park*

This is a report of four years' work on the control of vegetable insects with hand equipment on mixed vegetable plots. Two years were devoted to liquefied gas sprays to secure a better general purpose formulation and information on the performance of several insecticides. When technical insecticides were applied in a solution of liquefied gas and a volatile solvent such as methylene chloride, which was used in these experiments, a residue of the technical insecticide was left on the plant so that the results were not influenced by a diluent, emulsifier, or a nonvolatile solvent. One year's effort was devoted to a comparison of dust, spray, and aerosol for control of vegetable insects and one year's work included observations on effectiveness of various dilutions of sprays applied with hand equipment. Results of the effectiveness

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Table 1.—The results of six aerosol formulations applied to cabbage, 1952.

Insecticide and Per Cent Concentration	Per Cent Heads Injured			Average Number of Insects per Plot July 25		Cabbage Yield (All heads)	
	None	Slight	Severe	Flea Beetle	Cabbage Looper	Pounds	No. Heads
Toxaphene 2	81	10	8	0	4	110	70
Methoxychlor 5	1	39	60	0	10	111	80
Malathion 4	25	35	38	1	1	101	78
Methoxychlor 5, rotenone 1	26	55	18	0	5	119	77
Dilan 2, lindane 2	70	25	3	1	1	102	78
Methoxychlor 5, Dilan 2	39	51	9	0	2	126	84
Untreated	5	13	81	23	23	32	53
L.S.D. at 5% level	13			7	2[a]	30	

[a] Calculated on treatments other than untreated check.

of treatment are based primarily on crop yields though supplementary observations were made on phytotoxicity and insect populations.

1952 AEROSOL EXPERIMENTS.—Six aerosol formulations were tested in 1952. All formulations were made with 50% methyl chloride propellent. The remaining 50% of the formulation was composed of the designated insecticide and the necessary amount of methylene chloride to complete the formulations. Thus, formulation one, containing 2% toxaphene, required 48% methylene chloride and formulation two, containing 5% methoxychlor, required 45% methylene chloride. Seven vegetables—cabbage, tomato, cucumber, cantaloupe, snap bean, lima bean, and potato—were planted in garden plots 35×50 feet and all treatments were replicated on four plots in randomized block arrangement. The percentage of insecticides used in the formulations and the number of applications for each crop are given in tables 1 to 5.

The Golden Acre variety of cabbage was set in the field June 23. The first insecticidal treatments were made June 25 and were repeated weekly until August 7, a total of 7 applications. Counts of number of flea beetles (*Phyllotreta* spp.) and the cabbage looper (*Trichoplusia ni* (Hbn.)) were made 2 days before and again 2 days after the July 23 treatments. The imported cabbageworm (*Pieris rapae* (L.)) was not abundant enough to give significant data. The first heads were harvested August 13, and the last on September 16. The results are given in table 1.

The Marketer variety of cucumber and the Seneca Bender cantaloupe were planted June 7; the first of 12 weekly treatments was applied June 19 and the last on September 9. Total yields from all plots of each treatment and observations on appearance and presence of melon aphids (*Aphis gossypii* Glov.) on August 31 are given in table 2.

Table 3.—Yields of snap and lima beans, 1952 garden aerosol experiment.

Insecticide and Per Cent Concentration	Pounds of Fruit	
	Snap Beans	Lima Beans
Toxaphene 2	117	140
Methoxychlor 5	103	148
Malathion 4	105	131
Methoxychlor 5, Cube 1	107	147
Dilan 2, lindane 2	102	142
Methoxychlor 5, Dilan 2	118	143
Check	40	135
L. S. D. at 5% level	28	ns

The Wade variety of snap bean was planted June 19; the first treatments were applied July 2, the last July 30, a total of 5. Fordhook 242 lima bean was planted the same time and received the first insecticidal treatments July 2. A total of 9 weekly applications of aerosol was made, the last on August 27. No insect population counts were made. The Mexican bean beetle (*Epilachna varivesta* Muls.) did not appear until the crops were nearly mature but the potato leafhopper (*Empoasca fabae* (Harr.)) was present during the growing period. Results of yields of fruit are given in table 3.

Tomatoes, Rutgers variety, were set in the field June 23; the first of the weekly applications of aerosol was made July 9, the last on September 27, a total of 14. Yields in number and weight of fruit and insect infestation on October 5 are given in table 4.

Katahdin potatoes were planted June 19 and received 12 weekly applications of all treatments. Yields and general appearance of the plots are given in table 5.

1954 AEROSOL EXPERIMENTS.—Six aerosol formulations were tested in garden plots, 18×30 feet each, which contained potatoes, snap beans, cucumbers and cabbage. Plots of each treatment were replicated six times in a random block arrangement. Yields were taken, observations were made on insect population, and the general appearance of all vegetables was noted in all plots. Formulations were all made with 50% propellent (methyl chloride), the

Table 2.—Results of the 1952 insecticidal aerosols on cucumber and cantaloupe.

Insecticide and Per Cent Concentration	Cantaloupe				Cucumber			
	Appearance	Aphid Infestation[a]	No. Fruit	Pounds Fruit	Appearance	Aphid Infestation[a]	No. Fruit	Pounds Fruit
Toxaphene 2	Poor	+	49	174	Poor	+	818	251
Methoxychlor 5	Good	++	86	407	Good	+++	1269	378
Malathion 4	Good	0	105	495	Fair	0	1200	379
Methoxychlor 5, Rotenone 1	Good	++	96	462	Good	+++	1751	525
Dilan 2, Lindane 2	Fair	0	57	230	Good	0	2176	748
Methoxychlor 5, Dilan 2	Poor	+	51	208	Good	+++	1776	553
Check	Good	++	97	465	Good	+++	1121	353
L.S.D. at 5% level			26	124			400	121

[a] *Aphis gossypii* Glov.

Table 4.—Results on tomatoes, aerosol experiments, 1952.

Insecticide and Per Cent Concentration	Insect Infestation,[a] October 5				
	Aphids	Fruitworm[b]	Hornworm[b]	No. Fruit	Lbs. Fruit
Toxaphene 2	severe	31	0	1879	705
Methoxychlor 5	severe	27	0	1820	686
Malathion 4	none	15	0	1681	644
Methoxychlor 5, rotenone 1	few	11	0	1835	703
Dilan 2, lindane 2	few	7	0	1644	661
Methoxychlor 5, dilan 2	severe	12	0	1784	660
Check	severe	41	6	1560	590
L.S.D. at 5% level		11		ns	ns

[a] *Macrosiphum solanifolii* (Ashm.), *Heliothis zea* (Boddie), and *Protoparce quinquemaculata* (Haw.), respectively.
[b] Total number of plants infested for all replicates, average 16 plants per plot; total of 48 plants for each treatment.

desired amount of insecticide, and the difference made up with methylene chloride as in 1952. Snap beans of the Top Crop variety received four applications of each treatment. Observations made on infestation of the strawberry spider mite (*Tetranychus atlanticus* McG.) and Mexican bean beetle and yields of beans are given in table 6.

Golden Acre cabbage received 10 applications of treatments. Yield results, the insect species present at time of harvest, and the degree of insect injury to the heads are given in table 7.

Katahdin potatoes received 10 weekly applications of all treatments while Marketer cucumbers received six weekly treatments. Yields of these two crops are given in table 8. No significant insect populations developed on these two crops.

Comparison of Methods of Application.—During the 1953 season malathion was applied by three methods: in a 4% dust with a rotary hand duster, in a 4% liquefied gas aerosol spray, and in a water spray at the rate of 1 quart of 55% emulsion concentrate to 100 gallons of water applied with a compressed air sprayer. All treatments were applied weekly, except for the second week in July at which time no treatments were made. The same vegetables were used and planted in small garden plots 35×50 feet as in 1952. Treatments were replicated six times in randomized block arrangement. The total amounts of dust, spray, and aerosol applied, the average rates of application, and the actual amount of malathion applied

Table 5.—Results on potatoes, garden aerosol experiments, 1952.

Insecticide and Per Cent Concentration	October 5		Pounds Tubers
	General Appearance	Aphid Populations[a]	
Toxaphene 2	Fair	None	206
Methoxychlor 5	Poor	None	218
Malthion 4	Fair	None	218
Methoxychlor 5, rotenone 1	Fair	Few	223
Dilan 2, lindane 2	Good	None	243
Methoxychlor 5, Dilan 2	Fair	None	259
Check	Poor	Few	217
L. S. D. at 5% level			ns

[a] *Macrosiphum solanifolii* (Ashm.).

Table 6.—Results on snap beans, 1954 garden aerosol experiments.

Insecticide and Per Cent Concentration	Spider Mite Infestation[a] 8/23	Per Cent Plants Injured by Mexican Bean Beetle	Potato Leafhopper Nymphs per 20 Leaves	Beans (Lbs.)
Toxaphene 5, malathion 5	1.0	1.6	.5	154
Dilan 2, malathion 5	.3	0	0	145
Strobane 2, malathion 5	.3	13.0	.3	135
Strobane 2, pyrethrum 1[b]	1.0	6.1	.1	128
Malathion 5, pyrethrum 1, DDT 5	1.0	.5	.1	126
Malathion 5, pyrethrum 1	.8	.5	.5	142
Check	3.0	13.0	.8	97
L.S.D. at 5% level	.8	8.1	ns	18

[a] *Tetranychus atlanticus* McG. Estimated rating when 0=none, 1=few, 2=moderate and 3=severe infestation.
[b] Extract containing 20% pyrethrins used in all pyrethrum formulations.

per acre are given in table 9. Yields of all vegetables in total pounds and total number of fruits for all replicates of each treatment are given in table 10. The average percentage of cabbage free of insect injury at harvest time was 90% on dusted plots, 10% on sprayed plots, 45% on aerosol plots, and zero on untreated plots. Two per cent of dusted heads, 17.2% of sprayed heads, 14.0% of aerosoled heads, 35% of untreated heads were infested with aphids. The percentages of snap bean pods at harvest showing scars resulting from insect injury were 0.04 from dusted plots, and 0.11 for each of the other treatments and the check plots.

Spray Dilution Experiments, 1955.—Since recommendations for dilution of insecticides for small amounts of finished spray appear to be based on the rate used in 100 gallons of water per acre, this experiment was designed to determine comparative effectiveness of more concentrated solutions. Malathion emulsion was applied at three concentrations, 1.5 pints of 55% emulsion concentrate in 100 gallons, in 50 gallons, and in 25 gallons. Two additional insecticide treatments were included in this experiment, a concentrated pyrethrum spray of 2% pyrethrum extract at the rate of 1 quart in 25 gallons of water and DDVP at the rate of 1 pound (actual) in 100 gallons of water. Each plot contained potatoes, tomatoes, lima beans, and cucumbers in single 25 foot rows. All treatments were replicated four times in randomized blocks. Records of the amounts of finished spray applied in each treatment were totaled for the season and were adjusted to amounts applied per acre (Table 11).

Because of adverse weather, extremely dry till late summer, followed by extremely wet conditions, only limited information was obtained on the effectiveness of the treatments. This information is summarized in table 12.

Discussion.—Of 12 aerosol formulations used in these experiments, three were for the purpose of observing the insecticidal efficiency of malathion, toxaphene, and methoxychlor; nine were formulated to find a satisfactory, generally effective combination. Formulations containing toxaphene and Strobane were extremely toxic to cucurbits and do not appear suitable for use in a general purpose aerosol. Methoxychlor and pyrethrum appeared mild in their action against insects and are not promising insecticides for a general purpose, aerosol formulation. Considering factors of phytotoxicity, toxicity to warm blooded animals and effectiveness against insects from

Table 7.—Results of 1954 garden aerosol treatments on cabbage given in totals for all replicates of each treatment.

Insecticide and Per Cent Concentration	No. Heads Injured			No. Heads Infested with		Yield of Cabbage	
	None	Slight	Severe	Cabbage Looper	Aphid[a]	Pounds	No. Heads
Toxaphene 5, malathion 5	70	3	1	1	0	109	74
Dilan 2, malathion 5	39	38	6	2	0	96	83
Strobane 2, malathion 5	50	19	2	0	2	93	71
Strobane 2, pyrethrum 1	43	27	11	5	8	128	81
Malathion 5, pyrethrum 1, DDT 5	60	15	3	1	0	104	78
Malathion 5, pyrethrum 1	21	42	24	5	0	117	87
Check	0	6	71	13	26	78	65
L.S.D. at 5% level	35			4	10	29	ns

[a] *Brevicoryne brassicae* (L.)

Table 8.—Yields of potatoes and cucumbers, garden aerosol experiments, 1954.

Treatment and Per Cent Toxicant	Pounds Potatoes	Cucumbers	
		Number	Pounds
Toxaphene 5, malathion 5	119	156	84
Dilan 2, malathion 5	113	1448	772
Strobane 2, malathion 5	139	889	477
Strobane 2, pyrethrum 1	115	770	407
Malathion 5, pyrethrum 1, DDT 5	107	1232	670
Malathion 5, pyrethrum 1	139	1418	742
Check	129	1491	795
L. S. D. @ 5% level	ns	347	192

Table 9.—Total amounts of dust, spray, and aerosol applied during the 1953 experiment with amount of actual toxicant by each method.

Method of Application	Total Amount		Average Amount Applied per Acre per Application	
	Applied	Toxicant (Lb.)	Preparation	Toxicant (Lb.)
Dust	156.5 lb.	6.26	68.9 lb.	2.75
Spray	119.2 gal.	1.89	52.5 gal.	.83
Aerosol	39.5 lb.	1.58	17.4 lb.	.69

results reported here and previously reported (Owens & Ditman 1950, 1952), lindane, Dilan, and malathion are the most promising materials for use in a general formulation about the home and garden. A lindane-malathion combination is extremely desirable but a slight off-flavor in some vegetables results. Just how much importance should be given this factor is difficult to determine. All

Table 11.—Insecticide treatments, dilution, and rate of spray application and actual insecticide applied on an acre basis to 1955 vegetable plots.

Insecticide	Dilution		Rate of Application per Acre		
	Insecticide	Water (Gal.)	Spray (Gal.)	Pints of Insecticide	Actual Toxicant (Lb.)
Malathion E.C.	1.5 pts.	25	34.3	2.05	1.28
	1.5 pts.	50	30.3	.90	.56
	1.5 pts.	100	31.2	.31	.29
DDVP	1.0 lb.[a]	100	28.6	—	.28
Pyrethrum E.C.	2.0 pts.	25	28.9	1.15	.02

[a] Alcoholic solution prepared by W. F. Barthel, Pesticide Chemicals Section of the Agricultural Research Service, U. S. Department of Agriculture, provided in amounts to make 2 gallons of spray at a mixing.

vegetables, except potatoes, from all of the experiments were eaten by a large number of people, and with no indication of off-flavors being present. In the laboratory off-flavors were detected in lima beans, tomatoes, and potatoes (unpublished results); only in potatoes was the off-flavor pronounced and objectionable. For use on vegetables Dilan appears to be the most efficient substitute for lindane.

The problem of concentration is extremely important not only for aerosols but for water sprays and dusts, as well. In experiments comparing performance of malathion dust, sprays, and aerosol, and sprays of various concentrations of malathion, insecticidal efficiency tended to vary directly with the amount of actual malathion applied. Since the rate of application varies for different operators the problem is not simple. Results reported here (table 9) indicate that a 4% malathion aerosol formulation does not deposit enough toxicant per acre particularly when compared with 4% dust. Early insecticidal

Table 10.—Yield of vegetables in the 1953 experiments, treated with malathion spray, dust, and aerosol.

Malathion	Cabbage		Cucumber		Cantaloupe		Tomato		Total Pounds		
	No. Heads	Total Pounds	No. Fruit	Total Pounds	No. Fruit	Total Pounds	No. Fruit	Total Pounds	Lima Bean	Snap Bean	Potatoes
Dust 4%	131	312	984	462	125	283	5583	1177	109	175	244
Emulsion 1 qt./100 gal. water	139	260	789	369	108	237	5432	1220	79	143	225
Aerosol 4%	133	281	722	319	101	209	5278	1085	76	132	220
Check	130	160	880	421	129	293	4959	1102	86	127	188
L.S.D. at 5% level	ns	23.5	ns	ns	ns	ns	ns	ns	9	13	ns

Table 12.—Results of treatments applied in the 1955 garden spray experiments.

Insecticide and Amount in Gallons Indicated	Lima Beans				Cucumbers	Potatoes
		Per Cent Leaves Injured By				
	Fruit per Plot (Lb.)	Mexican Bean Beetle	Bean Leaf Beetle[a]	Spider Mite[b]	Fruit per Plot (Lb.)	No. Colorado Potato Beetles[c] per Plot
Malathion, 1.5 pts./25	5	1	3	3	35	2
1.5 pts./50	4	3	3	3	28	44
1.5 pts./100	4	14	31	14	24	77
DDVP, 1 lb./100	4	16	6	8	25	36
Pyrethrum, 1 qt./25	3	13	22	9	22	8
Check	2	70	100	20	27	20

[a] *Cerotoma trifurcata* (Forst.).
[b] *Tetranychus atlanticus* McG.
[c] *Leptinotarsa decimlineata* (Say).

aerosol formulations were designed for use in closed spaces where insecticide particles suspended in air were present for varying periods; the amount of insecticide was, therefore, kept at a minimum in the formulations. Such formulations may be regarded as space formulations. For successful use on garden plants, aerosols must deposit insecticidal residues comparable to those obtained with dusts and sprays if they are to be equally effective. These may be designated as residual formulations and to be effective should contain more actual insecticide than space formulations. The dilution of water for hand application sprays poses a similar problem which is not easy to overcome since the amount of spray applied varies with individuals, in these experiments from about 30 to 50 gallons per acre (tables 9 and 11).

Summary.—Of the several insecticides observed in 12 aerosol formulations, lindane, Dilan, and malathion appear most suitable for use in residual aerosols for use about the yard and garden. A combination of malathion with Dilan or lindane is suggested. The effectiveness of malathion in dust and in water sprays and aerosols at varying concentrations varied with the amount of actual insecticide applied. It appears from these studies that residual aerosols should contain a higher percentage of toxicant than usually formulated in space aerosols. DDVP showed promise against insect pests and mites on beans.

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Methods and Materials for Controlling Budworms on Flue-Cured Tobacco[1]

F. E. Guthrie,[2] R. L. Rabb,[2] R. L. Baron,[3] and J. E. Flynn[3]

Budworms, primarily *Heliothis virescens* (F.) and to a lesser extent *H. zea* (Boddie), are among the most important pests of flue-cured tobacco in North Carolina. These insects are among the most difficult to control on tobacco prior to topping because a high proportion of the larvae are sheltered within the tobacco bud, where it is difficult to place an insecticide. Continuous infestation coupled with the rapidity of growth of the tobacco bud further complicates control. The majority of growers in the state attempt to control these pests with dusts applied to the top of plants with a sack or perforated can or with dusts or sprays applied with conventional high clearance equipment.

Tests were conducted in 1955 and 1956 to determine the comparative effectiveness of insecticides, methods of application, dosage rates, spray volumes, and nozzle arrangements on control of budworms.

Review of Literature.—For many years lead arsenate-corn meal bait was the most effective means of budworm control (Morgan & McDonough 1917). With the advent of the synthetic organic insecticides, Allen & Early (1947) showed DDT to be a very effective material for controlling these pests. They also compared various methods of applying DDT and found a puff duster to be the most satisfactory method. Hand and shaker bottle applications of DDT dust were also very effective, and control with a rotary hand duster was fairly effective. Sprays applied with mule-drawn sprayers at the rate of 3 pounds of DDT using 75 gallons of spray per acre failed to give satisfactory control. Sprays applied with a knapsack sprayer at about 0.2 lbs. per acre of DDT gave good

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[2] Research Assistant Professor, North Carolina State College, Raleigh.
[3] Graduate Assistant.

plant protection but was inferior to control with a puff duster. Kuitert & Tissot (1949) and Chamberlin (1954), using rotary hand dusters, confirmed the results with DDT and also found that TDE was a promising material. Recently, Gast *et al.* (1956) investigated the comparative topical toxicity of a number of insecticides to both species of budworms and found that endrin was markedly superior to the other chlorinated hydrocarbon insecticides.

METHODS.—*1955 Tests.*—Plots were artificially infested by placing budworm eggs or first instar larvae on the buds of each plant thus insuring a uniformly heavy infestation. In test 1, one egg or larva was placed in each bud with the result that approximately 50% of the plants were infested at the time of treatment. In test 2, two eggs were placed in each bud, and the infestation at treatment was about 90%.

Tobacco was 45 to 60 days old and varied from 2 to 5 feet in height. All application methods directed the material toward the bud regardless of plant height. The single row plots were 30 plants long, replicated three times, and separated from adjacent plots by two to four guard rows depending on danger of contamination. The treatments were applied 4 or 5 days after infestation was established. The method, material and rates of application are shown in table 1. The pinch method involved placing a pinch of dust or bait into each bud. The rotary hand dusters delivered a continuous flow of dust above the plants. In the shaker can method, a perforated can containing insecticide dust was shaken over each bud. A Hudson compression sprayer, which delivered 12 gallons of spray material per acre at 40 p.s.i. through a solid cone nozzle directly to the bud, was used for the hand sprayer treatments. The power sprayer used was a Hahn Hi-Boy which applied 12 gallons of spray per acre through a single hollow-cone nozzle which was held 1 to 2 feet above the buds. The setting water treatment was applied at the rate of 200 gallons of treated water per acre by means of a hand setter. At 3 and 8 days following treatment, 20 plants in each plot were examined to determine the number of budworms and extent of damage. The following damage rating system was used:

0—(clean)—no damage
1—(light)—one or more leaves showing definite budworm injury, but no leaf with over one-tenth of the leaf consumed.
2—(moderate)—bud not damaged but one leaf one-fourth to one-half consumed *or* three leaves one-tenth to one-fourth consumed.
3—(heavy)—bud badly damaged *or* one leaf one-half consumed *or* two to three leaves one-fourth to one-half consumed *or* four to five leaves moderately damaged.
4—(severe)—bud badly damaged *and* either two leaves one-half or more consumed *or* four or more leaves one-fourth to one-half consumed.

1956 Tests.—The size of plants, method of infestation, extent of guard rows, and categories of plant injury were identical to those in the 1955 tests. However, in the 1956 tests the plots were replicated four times, and 25 plants in each plot were examined for larvae and injury.

In one experiment a split plot design experiment was conducted in which various application methods were compared 1 to 5 days after infestation. The methods of application included most of those used in the 1955 test except that a different nozzle arrangement was used with the mechanical sprayer, and the sack method was sub-

Table 1.—Comparative effectiveness of insecticides and application methods on reduction of tobacco budworms and damage, 1955.

MATERIAL	METHOD OF APPLICATION	POUNDS ACTUAL PER ACRE	NO. LARVAE/20 PLANTS (DAYS AFTER TREATMENT)		DAMAGE CATEGORY[b]
			3	8	
		Test 1			
1.5% endrin dust	Pinch/bud	0.09	4.3	0.0	8
DDT-corn meal bait, 1-75	Pinch/bud	.14	3.7	0.0	11
Lead arsenate-corn meal bait, 1-75	Pinch/bud	.14	6.7	1.0	11
1.5% endrin dust	Rotary hand duster	.12	3.3	0.0	11
10% TDE dust	Shaker can	.60	4.7	2.0	14
10% DDT dust	Rotary hand duster	.80	5.7	2.7	15
10% DDT dust	Shaker can	.60	7.7	2.7	15
40% Thimet W. P.	Transplant water[a]	.25	7.3	5.0	27
Check	———	—	11.0	6.0	35
L.S.D. at 5% level			4.0	2.2	9.0
at 1% level			5.4	2.9	12.0
		Test 2			
19% endrin E. C.	Hand sprayer	0.20	3.0	0.0	24
1.5% endrin dust	Rotary hand duster	.15	5.3	0.7	24
DDT-corn meal bait, 1-75	Pinch/bud	.09	7.0	1.3	25
19% endrin E. C.	Hand sprayer	.05	4.7	1.3	27
25% TDE E. C.	Hand sprayer	.19	8.3	1.3	31
10% DDT dust	Rotary hand duster	1.40	9.0	3.3	34
25% DDT E. C.	Hand sprayer	.19	10.3	1.0	38
Lead arsenate-corn meal bait, 1-75	Pinch/bud	.14	13.7	4.7	40
25% DDT E. C.	Power sprayer	.63	18.0	6.0	48
Check	———	—	24.7	15.3	55
L.S.D. at 5% level			6.3	2.1	10
at 1% level			8.5	2.8	14

[a] 2½ oz. per 50 gallons of water at 200 gallons per acre.
[b] Total for 20 plants/replicate.

Table 2.—Effect of different materials and application methods on tobacco budworms and injury, 1956.

Material	Method of Application	Pounds Actual per /A.	No. of Larvae/25 Plants[a] (Days After Treatment) 3	8	11	Damage Category[c]	Pounds Actual per Acre	No. of Larvae/25 Plants[b] (Days After Treatment) 2	6	Damage Category[c]
10% TDE dust	Pinch/bud	0.70	4	1	1	12	0.36	1	0	44
25% DDT E. C.	Hand sprayer	.20	3	1	3	9	.20	2	1	41
10% DDT dust	Puff duster	.60	3	3	2	15	.36	4	1	43
1.5% endrin dust	Rotary duster	.19	6	3	2	15	.21	4	1	45
DDT-corn meal bait, 1-75	Pinch/bud	.15	5	5	4	25	.10	1	0	43
10% DDT dust	Rotary duster	1.70	12	6	8	36	1.60	9	5	55
10% DDT dust	Sack	2.70	14	7	9	41	1.90	14	12	67
Lead arsenate-corn meal bait, 1-75	Pinch/bud	.15	16	13	11	48	.17	6	2	48
18.5% endrin E. C.	Mechanical sprayer[d]	.04	16	11	11	54	.04	5	4	48
25% DDT E. C.	Mechanical sprayer[d]	.20	21	13	12	50	.20	7	5	52
Check	———	—	30	20	18	80	—	24	21	75
L.S.D. at 5% level			6	4	4	12		5	3	10
at 1% level			8	5	6	16		6	4	14

[a] Applied to eggs and first instar larvae.
[b] Applied to third and fourth instar larvae.
[c] Total for 25 plants/replicate.
[d] Three solid cone nozzles/row delivering 35 gals./acre.

stituted for the shaker can method. In the sack method, a cheese cloth bag containing insecticide dust was shaken over the bud of each plant. In addition, a plunger type (puff) hand duster which directed a puff of dust into each bud was included.

In a second experiment, mechanical sprayers were used to test the effect of different nozzle arrangements, spray patterns, and spray volumes on control of larvae 4 days after infestation. The nozzles were adjusted so that the maximum amount of spray was delivered to the bud regardless of nozzle number and arrangement. The nozzles were placed 22 inches apart on a horizontal boom. In the final experiment, the effect of different concentrations of DDT using mechanical sprayers was evaluated 2 days after infestation.

All the specimens reared in the 1955 and 1956 tests were *H. virescens*, but this does not preclude the possibility of a small percentage of *H. zea* being present.

Results and Discussion.—In test 1 (table 1), all treatments, with the exception of Thimet applied in the transplanting water, resulted in fewer larvae and less damage than in the untreated check. On the basis of the number of larvae present 8 days after treatment, a pinch of DDT-corn meal bait to each bud was significantly better than 10% DDT dust applied either with a shaker can or a rotary hand duster. A pinch of 1.5% endrin dust

Table 3.—The use of a high clearance sprayer comparing type and number of nozzles and gallonage of DDT[a] on second and third instar tobacco budworms, 1956.

Spray Pattern	Pressure (p.s.i.)	No. Nozzles per Row	Gallons Spray Delivered /Acre	No. Larvae/25 Plants (Days After Treatment) 3	5	12	Damage Category[b]
Fan	60	3	9	21	15	5	72
Fan	60	3	18	21	13	4	73
Fan	60	3	28	17	12	5	65
Solid cone	60	3	35	23	12	5	72
Solid cone	60	3	51	17	10	3	62
Hollow cone	60	3	9	25	17	5	79
Hollow cone	60	3	18	21	15	8	75
Hollow cone	60	3	28	21	10	5	72
Hollow cone	35	3	26	22	13	6	70
Hollow cone	95	3	34	22	12	5	66
Fan	60	1	11	22	15	5	74
Fan	60	1	33	25	13	5	67
Hollow cone	60	1	11	24	13	5	74
Solid	60	1	15	22	17	7	73
Solid cone[c]	40	1	13	7	1	1	23
Untreated	—	—	—	28	18	8	83
L.S.D. at 5% level				6	4	3	12
at 1% level				8	5	4	16

[a] Applied at 0.2 pound per acre.
[b] Total for 25 plants/replicate.
[c] This treatment applied with a hand sprayer, all other treatments applied with a high clearance sprayer.

Table 4.—Effect of dosage rates of DDT applied with high clearance sprayers, using 3 nozzles per row, against first and second instar tobacco budworm larvae, 1956.

Pounds per Acre	Spray Pattern	Gallons Spray Delivered/Acre	No. Larvae/25 Plants (Days After Treatment)			Damage Category (Total for 25 Plants)
			3	5	7	
0.2	Solid Cone	51	16	15	13	50
0.5	Solid Cone	51	13	14	10	37
1.0	Solid Cone	51	12	10	6	31
1.0	Hollow Cone	18	13	13	9	43
Check	———	—	23	22	17	67
L.S.D. at 5% level			6	6	6	17
at 1% level			8	8	9	24

to each bud was as effective as the same material applied with a rotary hand duster. The lead arsenate bait and 10% TDE dust were not significantly less effective than the best treatments.

All treatments in test 2 (table 1) resulted in a lower infestation than in the untreated check except DDT applied with a power sprayer. Although the latter treatment resulted in fewer larvae, it failed to reduce damage significantly below that found in check plots. When applied with a hand sprayer, endrin, TDE, and DDT gave similar control of budworms on the basis of living larvae present 8 days after treatment. However, 1.5% endrin was significantly more effective than 10% DDT when applied with a rotary hand duster. DDT bait gave excellent results and was significantly more effective than the lead arsenate bait.

In 1956 the trends shown in the previous year were further investigated. Where treatments were applied immediately after infestation, the application methods (table 2) which placed insecticide directly in the bud gave much better results than the more indirect methods except that, as in 1955, the endrin dust treatment was among the better treatments. The DDT bait again gave better control than the lead arsenate bait. Endrin applied with a rotary hand duster was significantly better than DDT applied in the same manner. The sack method resulted in only moderate control of budworms, and the mechanical sprayer treatments were among the least effective treatments.

Where application was delayed until 5 days after infestation, the lead arsenate bait and the power sprayer applications gave comparatively better results. This may have been due to the fact that the larger larvae sometimes leave the bud and thus have a greater chance to come into contact with an insecticide. Other treatments performed essentially as they did against eggs and first instar larvae. As to be expected, damage was much greater where application was delayed. This illustrates the great importance of proper timing in the prevention of budworm damage.

Table 3 presents data on the effect of nozzle number and arrangement, spray pattern, and spray volume in the control of budworms. Although the hand sprayer treatment was outstanding in the control of budworms none of the alterations of the mechanical sprayers approached commercial control. In general the fan and solid cone patterns seemed slightly more effective than the hollow cone patterns, three nozzles gave better control than one nozzle, and higher spray volumes were better than low volumes. Even these trends were not always significant or consistent. Many of the treatments were not significantly different from the check.

Since low dosage might have been one of the reasons for unsatisfactory results with power equipment, the test, results of which are shown in table 4, was made. This experiment showed that although the trend was toward better results with higher dosages, the control was still below that desired. Again, as in table 3, a solid cone seemed to be better than a hollow cone pattern although the difference was not significant.

The poor results given with power sprays are probably explained by the morphology of the tobacco bud. The tip stem region ("bud"), composed of the apical meristem and the unfolded leaves, is protected by two unfolding leaves which overlap the so-called "bud." Therefore, unless these leaves are held or pushed aside by force they

FIG. 1.—Diagrammatic sketch of side view of bud region. 1. Showing bud protected from above by unfolding leaves; 2. Same, with one leaf pulled aside to expose bud from above.

appear to prevent the entrance of practically all of the material directed at the "bud" (see fig. 1).

As many growers in North Carolina are using the sack, shaker can, or mechanical equipment methods for controlling budworms, it is unlikely that control of severe outbreaks would be as effective as desired. Such a situation occurred in the 1956 season, and numerous complaints of "resistant" budworms were received. The most frequent complaints were heard (and observed) following the use of mechanical dusters, sprayers, and airplane equipment.

It seems well to speculate that if a grower does not wish to use methods of direct application to the bud he might apply insecticides frequently on a preventive basis and get satisfactory results. Eggs are not generally laid on the bud leaves, and it takes about 24 hours for the young worms to reach protection (Morgan & McDonough 1917). In this case the sack method or power equipment would probably be satisfactory application methods if applied frequently. These methods would also be effective against hornworms (*Protoparce* spp.) if present.

SUMMARY.—Comparisons of various application methods, materials, nozzle arrangements, and dosage rates to control tobacco budworms (*Heliothis* spp.) were investigated. It was found that when the insecticide was applied directly into the bud the best control was attained. The sack and shaker can methods were ineffective. Endrin was superior to DDT or TDE in controlling budworms with rotary hand dusters. A DDT bait was found superior to a lead arsenate bait. Although excellent results were obtained when hand sprayers were used, results with mechanical sprayers proved unsatisfactory regardless of nozzle arrangements, gallonage applied, pressure used, or dosage of insecticide. Since most of the tobacco is treated with mechanical equipment or by the sack method for budworm control, growers should put more emphasis on directing the insecticide into the bud.

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Further Observations on the Effect of Biting Fly Control on Milk Production on Cattle[1,2]

PHILIP GRANETT and ELTON J. HANSENS, *Rutgers University, New Brunswick, N. J.*

A report (Granett & Hansens 1956) of a limited series of trials during 1955 has already indicated that control of biting flies on cattle increases milk production and is a profitable procedure. Control of flies was accomplished with water-emulsifiable sprays containing methoxychlor with or without butoxy polypropylene glycol (Crag Fly Repellent). In an attempt to give these findings greater validity, the work was repeated in the same locality, Salem County, New Jersey, during the 1956 season on three herds using a weekly or semiweekly schedule. On a fourth herd, wettable powders containing the same active ingredients as the emulsifiable treatments, were applied dry or dispersed in water.

PROCEDURES AND MATERIALS.—In general, these were similar to those already reported (Granett & Hansens 1956). The cows were numbered and preliminary observations were made in the field so that each herd could be divided into three groups having approximately equal milk production and fly susceptibility. Biting flies were counted on the entire animal once per day, Monday through Friday. Daily milk weights were recorded for morning and evening milking.

The formulations and treatments for the sprays were:

Group A: A concentrate containing 10% methoxychlor, 8.8% Emcol H-145, and 81.2% Mobilsol 544-C was diluted 1 to 19 parts water and approximately 1 quart applied per animal.[3] Thus, the diluted spray contained 0.5% methoxychlor.

Group B: A concentrate (F-21) containing 5% methoyxchlor, 50% butoxy polypropylene glycol, 4.4% Emcol H-145, and 40.6% Mobilsol 544-C was diluted 1 to 9 parts water, and approximately 1 quart applied per animal.[3] Thus the diluted spray contained 0.5% methoxychlor plus 5.0% butoxy polypropylene glycol.

Group C: Untreated.

The formulations and procedures for the wettable powder treatments were:

Group A: 50% methoxychlor wettable powder.[4]

Group B: 50% methoxychlor plus 10% butoxy polypropylene glycol wettable powder.[3]

Groups A and B were treated with dry applications at the rate of 7.6 gms. per animal, and sprays at the rate of 8 lbs. per 100 gallons water, 1 quart per animal.

Group C: Untreated.

On the day the cows were treated at any of the farms, all Group C (untreated) animals were released from the barn after they were milked. Group A animals were then treated and released, and then Group B animals were treated.

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[2] Investigations conducted under research grants sponsored by Carbide and Carbon Chemicals Company, and E. I. duPont de Nemours and Company at Rutgers University.

[3] Carbide and Carbon Chemicals Company formulation.

[4] E. I. duPont de Nemours and Company formulation.

Farm 1 (Richie) and Farm 2 (Haynes), where tests were also made in 1955, are located in the lowland along the Delaware Bay Shore. Cows on these farms were usually attacked not only by biting flies, but also by several species of mosquitoes, mostly from the salt marsh. Farm 1 contained five Holstein cows per group which were treated with diluted emulsion concentrates once a week, usually on Monday morning. Farm 2 contained four Guernsey cows per group which were treated twice per week, usually Monday afternoon and Thursday morning or afternoon. Farm 3 (Grier) was located 6 miles from these farms, away from the low-lying land and further from the source of mosquito breeding. The herd was divided into groups of six Holstein cows each. The same treatments as at Farm 1 were applied once a week on Tuesday mornings to the cows on Farm 3. The herd on Farm 4 (Richman), located across the road from Farm 3, also was divided into groups of six Holsteins. Here, however, the wettable powder dusts were applied by hand to the backs and sides of the cows once a week, usually Monday mornings, during the first 4 weeks. The dusting tests were terminated the week of July 10. On July 26, spraying with dispersions of the wettable powders was begun and continued on four Holstein cows in each group for the following 4 weeks. These sprays were applied once per week, usually on Thursday afternoons.

Except for early in the season, flies were less numerous in 1956 than in the previous year. They developed rapidly in the spring, and by mid-June there were many more horn flies (*Siphona irritans* (*L.*)) than at the same time in 1954 or 1955. In July stable flies (*Stomoxys calcitrans* (*L.*)) began to be more abundant. Despite adequate rainfall the lower than normal temperatures which prevailed throughout the season appeared to slow breeding of these species as well as the species of horse flies (*Tabanus spp.*) and deer flies (*Chrysops spp.*) normally present in moderate numbers during June and July.

In addition to biting flies, mosquitoes were an important source of annoyance to animals and no doubt were a factor in milk production. It was not possible to make counts of mosquitoes attacking the various groups of animals, but New Jersey mosquito light traps were operated every night at three of the farms. In the 6 weeks beginning June 15 nearly 6,000 mosquitoes were taken in the trap at Farm 1, and 9,000 in the trap at Farm 2 located near the barns. An indication of the attraction of mosquitoes to cattle was obtained at the latter farm where a second trap was operated at the house located about 200 feet from the barn. Here only 3,500 mosquitoes were taken. At Farm 4, which is located some distance from large mosquito breeding areas, less than 500 mosquitoes were taken in the trap. Farm 3 was located directly across the road from Farm 4 and consequently no trap was operated there. The mosquitoes which were prevalent consisted of 51.7% *Culex* spp. (*C. pipiens* L. and *C. salinarius*), 16.9% *Aedes sollicitans* (Wlk.), 17.4% *Aedes cantator* (Coq.), and 11.0% *Aedes vexans*.

RESULTS AND DISCUSSION.—*Weekly applications.*—Farms 1 and 3 will be considered together because the herds were treated once a week in the same way with the diluted emulsion concentrate formulation. A summary of the average number of biting flies per cow per day for each group, and for 5 days in the week during the 6-week period is presented in table 1 for Farms 1 and 3. A similar summary of milk production for 7 days in the week on these two farms is presented in table 2. The data confirm the findings of 1955 in that the sprayed groups were significantly superior to the unsprayed group.

From the viewpoint of fly control, there was a tendency for the formulation containing butoxy polypropylene glycol and methoxychlor to be superior to the formulation containing methoxychlor alone. This difference between treatments was not significant, however.

The difference in control between treated and untreated groups, however, was sufficient to provide a substantial increase in milk on Farm 1. This amounted to about 36 pounds of milk per cow for an average week, and at the estimated price of 5 cents per pound, this would be $10.80 for a 6-week period. On the basis of $1.20 for the cost of "B" spray, this would net $9.60. This is considerably more than was obtained in 1955 because in 1956 there was a significant difference between treated and untreated groups on all days of the week, whereas in 1955 a significant difference was found on only 2 days. The cost of the "A" spray was only about 25 cents per cow for the 6-week period. However, these cows produced less than the cows treated with the B formulation and consequently the return from the extra milk was about the same.

On Farm 3, which was farther away from the marshland, insect annoyance from biting flies and mosquitoes was not as great, and the difference between treated and untreated groups was not as strongly reflected in milk production. Again, the increase was most evident from the group sprayed with the butoxy polypropylene glycol plus methoxychlor. For the 4 days that a significant difference was shown, the increase in milk would yield the farmer approximately $3.50 per cow for the 6-week period when cost of the spray is deducted. Production from the A group was significantly different from the untreated on only 1 day, and the benefit from use of this spray was reduced to about 50 cents per animal.

Semiweekly spray.—Farm 2 on which the twice-a-week trials were run contained the smallest herd. Only four Guernsey cows per group could be selected, and during the first week one of the cows in the "C" group was sold. The figures obtained for fly control and milk production have been averaged for each week of a 4-week period and compared with data obtained prior to treatment. Table 3 gives this information for the number of flies observed and the percentage control, and for the milk production and difference noted within each group.

The breeding schedule on this farm was such that milk production generally declined during the summer months regardless of other conditions. It will be observed, however, that the milk production from the cows sprayed twice a week with the "B" formulation was least affected, whereas production from the "A" and "C" groups suffered significant decreases from that obtained originally. It is difficult to judge from this limited trial how much better a semiweekly schedule of applications is than a weekly treatment schedule. Fly control was excellent, and milk production was maintained on a high level, especially where butoxy polypropylene glycol was used with methoxychlor.

Wettable powder dry treatments.—On Farm 4 control of horn flies from wettable powders applied as dusts at the

Table 1.—Daily average number of flies counted on sprayed and unsprayed groups of cows during a 6-week period.

Days After Spray	Farm 1				Farm 3			
	Average Number of Flies/Cow/Day on Group			Difference Significant Between Groups	Average Number of Flies/Cow/Day on Group			Difference Significant Between Groups
	A	B	C		A	B	C	
0[a]	111	109	105	none	33.7	30.9	32.6	none
1	9.3	6.1	102.0	A, B vs. C	1.0	0.3	30.7	A, B vs. C
2	10.0	8.3	26.0	A, B vs. C	4.9	2.3	23.1	A, B vs. C
3	8.7	7.3	21.3	A, B vs. C	4.5	2.7	13.3	A, B vs. C
4	11.7	13.1	21.5	A, B vs. C	3.3	2.1	11.8	A, B vs. C
5	12.1	14.5	25.0	A, B vs. C	—	—	—	
7	—	—	—		14.1	10.0	29.7	A, B vs. C
Average/cow/day for 6 weeks	10.3	9.8	39.2		5.6	3.5	21.7	
Difference from untreated	−28.9	−29.4			−16.1	−18.2		

[a] Counts during week preceding first spray application.

Table 2.—Daily average milk production from sprayed and unsprayed groups of cows during a 6-week period.

Days After Spray	Farm 1				Farm 3			
	Daily Average Pounds of Milk per Cow on Group			Difference Significant Between Groups	Daily Average Pounds of Milk per Cow on Group			Difference Significant Between Groups
	A	B	C		A	B	C	
0[a]	32.1	30.4	31.0	none	33.3	32.6	32.6	none
1	26.6	27.0	22.2	A, B vs. C	30.5	31.1	28.5	none
2	26.9	27.1	22.2	A, B vs. C	31.1	31.5	29.1	none
3	27.3	28.1	23.0	A, B vs. C	31.5	32.5	28.0	B vs. C
4	27.1	27.8	22.2	A, B vs. C	31.3	32.1	28.7	B vs. C
5	27.0	26.9	21.9	A, B vs. C	30.0	31.0	27.3	B vs. C
6	27.5	27.5	22.5	A, B vs. C	30.1	31.5	27.5	A, B vs. C
7	26.8	27.7	21.9	A, B vs. C	30.0	31.3	28.1	none
Total/cow/week	189.2	192.1	155.9		214.5	221.0	197.2	
Difference from untreated	+33.3	+36.2			+17.3	+23.8		

[a] Milk production for 7 days during week preceding first spray application.

Table 3.—Average number of flies and milk production for groups of cows sprayed semi-weekly for a period of 4 weeks, Farm 2.

Time	Average Number of Flies and Per Cent Control per Cow per Day on Groups						Average Milk per Cow per Day and Per Cent Difference from Amount Prior to First Application on Groups					
	A		B		C		A		B		C	
	No.	Per Cent Control	No.	Per Cent Control	No.	Per Cent Control	Milk (Lbs.)	Per Cent Difference	Milk (Lbs.)	Per Cent Difference	Milk (Lbs.)	Per Cent Difference
Prior to spray	129	—	143	—	167	—	21.3	—	18.9	—	28.3	—
1st week	19.3	85	16.0	89	56.5	66	20.3	−4.7	18.9	0	25.7	−9.2
2nd week	20.2	84	15.8	88	24.4	85	18.9	−11.3	18.4	−2.6	24.3	−14.1
3rd week	17.5	86	13.0	91	19.2	88	17.5	−13.1	18.5	−2.5	23.5	−17.0
4th week	17.0	87	8.4	94	19.9	88	19.3	−9.4	18.7	−1.6	23.2	−18.0
L.S.D. at 5% level	28		20		34		2.1		2.2		2.3	

Table 4.—**Average number of flies and milk production for groups of cows treated with wettable powder dusts for 4 weeks and wettable powder sprays for 4 weeks (Farm 4).**

Time	Average Number of Flies and Per Cent Control /Cow/Day on Groups						Average Milk/Cow/Day and Per Cent Difference from Amount Prior to First Application on Group					
	A		B		C		A		B		C	
	No.	Per Cent Control	No.	Per Cent Control	No.	Per Cent Control	Milk (Lbs.)	Per Cent Difference	Milk (Lbs.)	Per Cent Difference	Milk (Lbs.)	Per Cent Difference
						Dusts						
Prior to dust[a]	154	—	142	—	161	—	27.0	—	25.9	—	27.3	—
1st week	12.6	92	9.2	94	33.8	79	30.7	+12.7	26.8	+6.1	28.3	+3.6
2nd week	15.0	90	13.6	90	24.8	85	30.7	+12.7	28.1	+8.8	29.1	+6.8
3rd week	14.0	91	13.8	89	29.0	82	30.1	+11.6	27.5	+7.7	28.3	+3.6
4th week	5.2	97	7.2	95	12.4	86	30.3	+12.1	27.1	+5.0	27.8	+2.1
L.S.D. at 5% level	31		24		48		1.8		1.8		1.5	
						Sprays						
Prior to spray[a]	34.6	—	42.0	—	54.4	—	36.6	—	29.4	—	36.4	—
1st week	6.5	81	5.8	86	8.8	84	36.4	−0.5	28.3	−3.7	35.5	−2.5
2nd week	6.5	81	4.4	89	8.2	84	30.6	−16.4	26.2	−10.8	34.3	−5.7
3rd week	3.0	91	2.0	95	4.8	91	32.6	−10.9	24.9	−15.3	32.9	−9.7
4th week	3.7	89	4.5	89	7.7	86	28.8	−21.3	24.2	−17.7	31.1	−14.5
L.S.D. at 5% level	7.1		6.9		11.2		1.4		1.4		1.4	

[a] Flies counted 5 days and milk production recorded for 7 days prior to first application of dust or spray.

rate of 1 tablespoonful per cow resulted in a marked reduction in fly population. This was apparent (see table 4) not only on treated groups, but also was noticeable on the untreated group.

The cows in this herd were near the peak of production, and during the 4 weeks that dusting continued, milk yield from all groups increased. This increase was most apparent from Group A, followed by Group B. Butoxy polypropylene glycol did not contribute to methoxychlor activity in the dust as it had in the emulsion concentrate formulation, partly because of the ratio of active ingredients, the method of application, and the habits of flies present. The dust, applied to the backs and upper sides of the animals where horn flies congregate, would not be as effective as sprays on stable flies which are habitually found on the legs and lower portions of the animals rather than on the back.

Wettable powder sprays.—The wettable powder sprays were started 2 weeks after the last dust application. The data for these treatments are presented in table 4. Flies were not as prevalent as at the beginning of the season, but when treatments were started, flies on all groups were immediately reduced to an average of six to eight, and finally to three or four per animal.

Because part of the herd was near or at the end of the lactation period, there were fewer cows available for testing on this farm, This made it necessary to reduce the number of cows per group from six to four. Unfortunately, with only four cows per group, there was no way to equalize the effect of an exceptionally high producer in the "C" group. The average of all groups was raised above that shown for these groups previously. Whether for these reasons, or because fly annoyance for the entire herd, including the untreated controls, was reduced below the point where it might affect production adversely, the increase in milk yield was no greater for the treated than for the untreated cows.

MILK ANALYSIS.—Milk samples for analysis to determine the presence or absence of methoxychlor were obtained at all farms. When results become available they will be published in a separate paper.

SUMMARY AND CONCLUSIONS.—Since promising results had been obtained on two farms in Salem County, N.J., in 1955, the tests were expanded in 1956 to establish more firmly the finding that control of biting flies can result in a significant increase in milk production. On two farms, water emulsion sprays were applied once a week, and on a third farm twice a week. At each location one-third of the herd was treated with a formulation containing methoxychlor, and one-third was treated with methoxychlor plus butoxy polypropylene glycol (Crag Fly Repellent) as the active ingredients. The other third of the herd was left untreated. On a fourth farm wettable powders of methoxychlor or methoxychlor plus butoxy polypropylene glycol were applied as dusts to groups of animals for 4 weeks. After a 2-week interval, the same groups were treated with sprays of the wettable powders. Daily observations were made of milk production and of the biting flies on individual cows in all herds.

On the two farms where one spray per week was applied, there was a marked difference between treated and untreated groups, both in reduction in fly annoyance and increased milk production. With twice-a-week application, results were similar, but not enough data was secured to determine whether the additional spray was warranted. Butoxy polypropylene glycol tended to increase the effectiveness of the formulation in which it was an ingredient. Greater increases in milk production re-

sulted from treatment of animals in 1956 than in 1955, and consequently greater monetary returns above the cost of the spray.

Both of the dust treatments were superior to no treatment, but in the formulation used butoxy polypropylene glycol did not appear to increase effectiveness of the methoxychlor. Excellent control of the fly population on the herd was obtained with applications of the wettable powder sprays. However, possibly because these tests were conducted later in the season when the horn fly population had been reduced, and when many of the cows were approaching the end of their lactation period, differences in milk production obtained by using the wettable powder sprays were not evident.

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Insect Control in Relation to Alfalfa Seed Production in Central Wisconsin[1]

J. T. MEDLER and G. N. BROOKS[2]

In a report by Medler (1955) on the control of alfalfa insects by several chlorinated hydrocarbon and phosphate insecticides, it was shown that a mixture of insecticides was more effective than these insecticides used alone. Additional tests were made in 1952, 1953, and 1954 in order to further evaluate certain mixtures of insecticides for the control of alfalfa insects, and also to determine the relations between insect control and alfalfa seed production.

METHODS.—The experiment was conducted on the Greenwood Game Refuge about 3 miles from the Branch Agricultural Experiment Station at Hancock, Wisconsin. The soil type of Plainfield sand had a C (=poor) agricultural rating. It was planned to use the same 65-acre field each year, but in the third year it was necessary to move the test to a 40-acre field about ½ mile away. Second-crop seed was produced each year.

Plots were each 5 acres in 1952 and 1953 and 3 acres in 1954. Insecticide emulsions were applied with a low-volume tractor sprayer at the preblossom stage of plant growth. The treatments were randomized each year. The entire plot was harvested with a combine and the seed re-cleaned once. The seed yields are given in table 1.

RESULTS.—All of the insecticide treatments except Systox and parathion gave significantly better yields than the untreated checks. There was no significant difference in the amount of seed produced by the various insecticide treatments. However, the DDT mixtures (7 treatments) gave better seed production than the individual insecticides (4 treatments). Over the three-year period the best treatment averaged 43.1 lbs. of seed per acre—the poorest 7.4 lbs.

Table 2 gives the counts of mirids, leafhoppers, and grasshoppers which were obtained by sweeping with a 15″ net on two dates. There was considerable variation from year to year and large differences between the good and poor treatments. The mirids and leafhoppers in the two untreated checks and parathion were not significantly different, and this can be explained on the basis of reinfestation following treatment with the non-residual insecticide. The DDT mixtures and DDT alone gave significant control of mirids. The DDT mixtures, DDT, and methoxychlor gave significant control of leafhoppers. All of the treatments except DDT gave a significant reduction of grasshoppers. The differences obtained between the various treatments appeared to be associated with (1), the clean-up of the grasshoppers, and (2), the residual protection against the mirids and leafhoppers given by the chlorinated hydrocarbons, especially DDT.

Table 1.—Pounds per acre of second-crop alfalfa seed obtained in plots at Hancock, Wisconsin.

TREATMENT[a]	1952	1953	1954	AVERAGE	MEANS[b]
DDT+heptachlor (c)	62.2	33.6	33.6	43.1	2.24
DDT+dieldrin (c)	46.4	41.5	35.3	41.1	2.24
DDT+chlordane	46.2	27.4	35.0	36.2	2.17
DDT+heptachlor	28.6	31.0	41.0	33.5	2.15
DDT+dieldrin	59.0	33.0	16.3	36.1	2.13
DDT+toxaphene	30.2	30.4	32.0	30.9	2.12
DDT+aldrin	24.4	31.0	38.6	31.3	2.12
Methoxychlor (c)	14.6	32.2	28.6	21.8	1.95
DDT	22.4	11.4	23.6	19.1	1.89
Systox (c)	8.0	21.2	13.3	14.2	1.75
Parathion (c)	2.4	28.0	22.3	17.5	1.70
Untreated check B	5.2	14.0	9.6	9.6	1.59
Untreated check A	.4	11.4	10.3	7.4	1.25
Average	26.9	25.9	26.2	26.3	

[a] The treatments are ranked according to decreasing effectiveness of means. Per acre dosages in lbs. tech. insecticide: methoyxchlor 1, parathion 0.25, Systox 0.25, DDT 0.5, aldrin 0.25, heptachlor 0.125, dieldrin 0.125, chlordane 0.5, toxaphene 0.75; (c) indicates commercial formulation, all others were laboratory mixes.

[b] Plot data transformed to log X+1. Analyzed by Duncan's multiple range test: Treatment means not underscored by the same lines are significantly different.

The control of the 3-insect complex was very important in relation to the alfalfa seed produced. Table 3 shows that significant correlation coefficients were obtained between the counts of the insects and seed yields in all comparisons except for leafhoppers and mirids in 1953. Best control and best seed yields were accomplished by using a mixture of DDT and any one of the following: aldrin, chlordane, dieldrin, heptachlor, toxaphene.

Relatively low yields of alfalfa seed were obtained in the plots with the best insecticide treatments. The fact that the seed produced per acre in all treatments averaged 26.9 lbs. in 1952, 25.9 lbs. in 1953, and 26.2 lbs. in 1954 indicates that some factor other than injurious insects was

[1] Accepted for publication January 5, 1957.

[2] Associate Professor of Agronomy and Entomology and former Assistant Professor of Branch Stations, respectively, University of Wisconsin. The authors thank personnel of the Wisconsin Conservation Department for their co-operation in this research.

Table 2.—Numbers of insects per 100 sweeps in alfalfa plots at Hancock, Wisconsin.

TREATMENT	1952[a]	1953[b]	1954[c]	TOTAL MEANS[d]	
MIRIDS[e]					
DDT+dieldrin (c)	53	14	20	87	1.41
DDT+dieldrin	63	13	35	111	1.51
DDT+heptachlor	124	25	27	176	1.66
DDT+heptachlor (c)	119	35	40	194	1.75
DDT+aldrin	158	39	28	225	1.75
DDT+toxaphene	134	23	55	212	1.75
DDT+chlordane	291	24	32	347	1.79
DDT	271	21	46	338	1.81
Systox (c)	474	34	63	571	2.01
Methoxychlor (c)	466	57	65	588	2.08
Untreated check B	194	108	154	456	2.17
Parathion (c)	678	11	84	773	2.18
Untreated check A	759	60	178	997	2.30
LEAFHOPPERS[f]					
DDT+toxaphene	42	2	62	106	1.30
DDT	45	2	69	116	1.33
DDT+aldrin	33	1	161	195	1.35
DDT+chlordane	60	1	142	203	1.41
DDT+heptachlor (c)	22	4	152	178	1.41
DDT+heptachlor	48	3	90	141	1.42
DDT+dieldrin	71	5	173	249	1.63
DDT+dieldrin (c)	50	18	184	252	1.75
Methoxychlor (c)	164	3	308	475	1.77
Systox (c)	97	13	376	486	1.90
Untreated check B	162	73	319	554	2.19
Parathion (c)	208	32	608	848	2.21
Untreated check A	319	22	712	1053	2.24
GRASSHOPPERS[g]					
DDT+dieldrin (c)	0	2	0	2	0.16
DDT+dieldrin	0	3	4	7	0.43
DDT+heptachlor (c)	0	6	2	8	0.44
DDT+aldrin	2	3	1	6	0.46
DDT+chlordane	1	4	3	8	0.53
Parathion (c)	1	5	12	18	0.73
DDT+toxaphene	1	3	21	25	0.75
DDT+heptachlor	7	10	3	20	0.83
Methoxychlor (c)	2	75	8	85	1.10
Systox (c)	10	149	6	165	1.34
DDT	18	280	73	371	1.87
Untreated check A	33	290	58	381	1.92
Untreated check B	31	285	102	418	1.99

[a] Treated July 9. Insects sampled July 16 and August 13.
[b] Treated July 25. Insects sampled August 5 and September 3.
[c] Treated July 9. Insects sampled July 13 and August 17.
[d] Transformed to log X+1. Analyzed by Duncan's multiple range test: Treatment means not underscored by the same lines are significantly different.
[e] *Adelphocoris lineolatus* (Goeze), 12.3%; *A. rapidus* (Say), 2.1%; *Lygus lineolaris* (P. de B.), 85.6%.: adults 54.4%, nymphs 45.6%.
[f] *Empoasca fabae* (Harr.): adults 71.5%, nymphs 28.5%.
[g] *Melanoplus* spp. including *bivittatus* (Say) *confusus* Scud., and *mexicanus* (Sauss.): adults 20.2%, nymphs 79.8%.

consistently limiting the seed yields.

Boron was not a limiting factor because the fields were top-dressed each spring with 0–9–27 B fertilizer. Incidentally, a narrow demonstrational strip was left unfertilized in 1952, and produced no seed where it crossed the 13 plots.

The total inches of rainfall in July and August was 6.56 in 1952, 5.50 in 1953 and 7.15 in 1954. Symptoms of moisture stress were not observed in the fields at any time. Therefore, inadequate moisture does not seem to have been a seriously limiting factor.

Probably the lack of pollinating insects was the major limiting factor for better seed production in association with the good control of injurious insects. The day-to-day pollination in the fields was not studied, but the number

Table 3.—Correlation analysis between seed yields and injurious insects.

CORRELATION	YEARS			AVERAGE 52+53+54
	1952	1953	1954	
Seed×leafhoppers	−.856[a]	−.300[n.s.]	−.640	−.809
Seed×mirids	−.786	−.500[n.s.]	−.822	−.870
Seed×grasshoppers	−.667	−.928	−.673	−.815
Seed×all three	−.892	−.804	−.519[n.s.]	−.954

[a] Value of correlation coefficient required to be significant for n=13 at 5% level=.553, at 1% level=.684.

Table 4.—Number of bees per 100 sweeps in alfalfa plots at Hancock, Wisconsin.

YEAR	HONEY BEES[a]	NATIVE WILD BEES[b]
1952	11.1	0.6
1953	1.5	0.4
1954	3.8	0.8
Average	5.5	0.6

[a] All appeared to be gathering nectar.
[b] *Bombus* spp. were 15%; others were *Lasioglossum* spp.

of bees obtained in the sweepings are recorded in table 4. A scarcity of pollinating insects was evident. The wild bees were predominantly *Lasioglossum* spp., which are believed not to be pollinators of alfalfa. Bumblebees were very scarce. Honeybees averaged 5.5 per 100 sweeps.

Grandfield & Franklin (1952) estimated that a population of one honeybee per square yard tripping 2% of the blossoms could produce 120 lbs. of alfalfa seed in a 20-day blooming period in Kansas. If it is assumed that one sweep of a 15″ net represented ¼ square yard, the average number of honeybees shown in table 4 was 0.2 per square yard. It was interesting to find that 0.2×120 lbs. gave 24 lbs., which approximated the 26.3 lbs. per acre overall average actually obtained. However, this close agreement is believed to be coincidental since the honeybee counts varied from 1.5 to 11.1 between the years, but the yields were about the same each year. Furthermore, honeybees were never observed tripping the alfalfa blossoms in the plots.

SUMMARY.—In a three-year large-plot test, alfalfa seed production was increased significantly by applications of insecticides. The yields of seed were correlated significantly with the insect control obtained. The injurious insect complex of leafhoppers-mirids-grasshoppers was controlled best by mixtures containing DDT and any one of several insecticides, including aldrin, chlordane, dieldrin, heptachlor and toxaphene.

Yields of seed were relatively low in plots which had good insect control. It is postulated that some factor other than injurious insects was consistently limiting seed production. Probably the most important was the lack of pollinating insects.

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Plant Metabolism of Dithio-Systox and Thimet[1,2]

ROBERT L. METCALF, T. R. FUKUTO, and RALPH B. MARCH, *University of California Citrus Experiment Station, Riverside*

The organo-phosphorus insecticide *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorodithioate, or dithio-Systox (produced by Farbenfabriken Bayer as compound 19639 or Di-Syston) is an obvious structural relative of the two Systox isomers, and Thimet (produced by the American Cyanamid Co. as compound 3911) is the closely related *O,O*-diethyl *S*-ethylmercaptomethyl phosphorodithioate. Both are systemic insecticides and appear to be especially useful for the treatment of seeds of cotton, alfalfa, sugar beets, flax and other agricultural crops to protect the newly emerged seedlings against the attack of such pests as mites, aphids, thrips, whiteflies, and caterpillars (Metcalf 1956).

The study of the plant metabolism of these compounds is an obvious extension of previous work from this laboratory in which it was demonstrated that the compounds *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate (Systox thiono isomer) and *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate (Systox thiol isomer) are metabolized in plant and animal tissues to the corresponding sulfoxide and sulfone derivatives, which are the ultimate systemic toxicants (Fukuto *et al.* 1955 and 1956, March *et al.* 1955, Metcalf *et al.* 1955).

MATERIALS AND METHODS.—This work employed radioactive tracers synthesized with P^{32} from neutron-irradiated red phosphorus.

P^{32}-Diethyl phosphorodithioic acid.—In a 50-ml. 3-necked flask equipped with stirrer, dropping funnel, and condenser was placed 4.0 gm. P^{32}-red phosphorus, 10.2 gm. sulfur, and 1.74 gm. phosphorus pentasulfide. Dry nitrogen was passed through the system, and the flask was heated in a silicone bath until the mixture melted. The mixture was then stirred until homogenous. The temperature of the bath was raised slowly to 280° C., maintained at that temperature for 5 hours, and then lowered to 80° C. After the addition of 15 ml. benzene, 13.2 gm. absolute ethyl alcohol was added dropwise. The mixture was heated at reflux until there was no further reaction (6 to 8 hours), filtered, and the P^{32}-labeled diethyl phosphorodithioic acid distilled; b.p. 50° C. (0.3 mm.); yield, 7.5 gm.

P^{32}-dithio-Systox (1).—To 0.57 gm. ammonium carbonate monohydrate in 20 ml. benzene was added dropwise 1.3 gm. P^{32}-diethyl phosphorodithioic acid, and the mixture heated until no more carbon dioxide was liberated. 2-Chlorodiethyl-sulfide (1.21 gm.) was then added, and the mixture was heated at reflux for several hours. The cooled benzene solution containing the product was washed twice with water, dried over anhydrous sodium sulfate, and the benzene was removed under vacuum. The P^{32}-dithio-Systox was not distilled.

The dithio-Systox was obtained as a product 99.9+% pure as evaluated by paper chromatography, and had a relative activity of 3.54 c.p.s. per γ by thin-window Geiger tube and 5.29 c.p.s. per γ by flow counter.

In order to demonstrate the oxidative metabolism of these compounds it was necessary to prepare the pure oxidation products to determine their properties and chromatographic behavior as shown in table 1. Compounds IV, V, and VI have been described previously (Fukuto *et al.* 1955).

O,O-Diethyl S-ethyl-2-sulfinylethyl phosphorodithioate (II).—To 10 gm. of *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorodithioate in 15 ml. acetone was added, dropwise, an equivalent amount of 30% hydrogen peroxide. The solution was kept at 50° C. for 1 hour, anhydrous sodium sulfate was added, and the mixture was allowed to stand for 3 days at room temperature. The mixture was then filtered and concentrated under vacuum. The oily residue was dissolved in benzene, washed several times with water, dried over anhydrous sodium sulfate, and distilled in the falling-film molecular still b.p. 150° (0.05 mm.). The yield was 5 gm. Analysis: calculated for $C_{18}H_{19}O_3PS_3$, C = 33.1%, H = 6.6%; found, C = 33.30%, H = 6.85%.

O,O-Diethyl S-ethyl-2-sulfonylethyl phosphorodithioate (III).—This compound was prepared according to the method described by Lorenz (1949), from 12 gm. vinyl ethyl sulfone and 18.6 gm. diethyl phosphorodithioic acid. Six grams of product were obtained by distilling at 170° (0.1 mm.) in the falling-film molecular still.

P^{32}-Thimet.—To 2 gm. P^{32}-diethyl phosphorodithioic acid in 15 ml. acetone was added, with stirring, 0.75 gm. anhydrous potassium carbonate. A slight excess of ethyl chloromethyl sulfide (1.3 gm.) was added in one portion, and the mixture was stirred for 2 hours at room temperature, filtered through celite, and concentrated under vacuum. The product was not distilled. The radioactive portion of the product was 99+% Thimet, as evaluated by paper chromatography, and had a relative activity of 5.79 c.p.s. per γ by thin-window Geiger tube and 8.40 c.p.s. per γ by flow counter.

O,O-Diethyl S-ethylmercaptomethyl phosphorothiolate.—To 30 gm. diethyl sodium phosphorothiolate in 100 ml. anhydrous toluene was added 17.3 gm. ethyl chloromethyl sulfide. The mixture was stirred and heated at 50° C. for about 2 hours. It was then cooled to room temperature, and water was added to dissolve the sodium chloride. The separated toluene layer was washed with water, dried over sodium sulfate, and the product was distilled, b.p. 110° (0.1 mm.), n_D^{30} 1.4925. Elemental analysis: per cent calculated for $C_7H_{17}O_3PS_2$, C = 34.41%, H = 7.02%; found, C = 34.52%, H = 7.29%.

O,O-Diethyl S-ethylsulfinylmethyl phosphorothiolate.—A mixture of 10 gm. *O,O*-diethyl *S*-ethylmercaptomethyl phosphorothiolate and 50 ml. chloroform was cooled in ice, and 190 ml. of a chloroform solution containing 6 gm. perbenzoic acid was added dropwise with stirring. The resulting mixture stood overnight in the refrigerator. It was washed twice with 5% sodium hydroxide and water, dried over mangesium sulfate, and concentrated to an

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oil. The oil was washed three times with Skellysolve B and distilled in the falling-film molecular still, b.p. 150° C. (0.2 mm.), n_D^{30} 1.4981. Elemental analysis: calculated for $C_7H_{17}O_4PS_2$, C = 32.26%, H = 6.59%; found, C = 32.90%, H = 6.90%.

0,0-Diethyl S-ethylsulfonylmethyl phosphorothiolate.—This compound was prepared in the same manner as the sulfinyl derivative described above, except that twice the amount of perbenzoic acid was used. The product was distilled in the falling-film molecular still, b.p. 170° C. (0.1 mm.), n_D^{30} 1.4863. Elemental analysis: calculated for $C_7H_{17}O_5PS_2$, C = 30.43%, H = 6.20%; found, C = 30.74%, H = 6.50%.

0,0-Diethyl S-ethylsulfinylmethyl phosphorodithioate.—This compound was prepared by oxidizing Thimet with an equivalent amount of perbenzoic acid, as described above for the corresponding phosphorothiolate. The product was distilled in the falling-film molecular still, b.p. 150° C. (0.1 mm.), n_D^{30} 1.5365. Elemental analysis calculated for $C_7H_{17}O_3PS_3$, C = 30.43%, H = 6.20%; found, C = 30.06%, H = 6.43%.

0,0-Diethyl S-ethyl-2-sulfonylmethyl phosphorodithioate.—This compound was prepared by oxidizing Thimet with two equivalents of perbenzoic acid, as described above for the corresponding phosphorothiolate. The product was distilled in the falling-film molecular still, b.p. 150° C. (0.1 mm.), n_D^{30} 1.5251. Decomposition occurred during the molecular distillation, and the product could not be purified to give proper elemental analysis. The infrared spectrum, however, showed the presence of the $-SO_2-$ (1320 and 1140 cm.$^{-1}$), P—O—C— (1020 cm.$^{-1}$) and P=S (655 cm.$^{-1}$) moieties.

All compounds described above were examined in the infrared region, and their spectra stand as further proofs of their structures. The infrared spectra were determined in a Perkin-Elmer model 21 recording spectrophotometer fitted with sodium chloride optics. Carbon tetrachloride was used as a solvent. Microanalyses were carried out by C. F. Geiger of Ontario, California.

Paper chromatography was accomplished by the reversed-phase system previously described (March *et al.* 1954), using 50% propylene-glycol-impregnated paper and a solvent of 1 part toluene and 4 parts hexane (Skellysolve B) saturated with propylene glycol. Under these conditions the R_f values for the oxidative compounds are as listed in table 1.

The study and isolation of metabolic products of the systemic compounds were carried out as follows. The bases of young cotton and lemon plants were treated with the P^{32}-labeled products either by making topical applications of 5 to 25λ to the bases of young cotton plants or 20λ to the bases of young lemon seedlings; or by cutting off the stems of mature cotton leaves and placing them in a water dispersion of the compound, using 3 parts of Thiosolve emulsifier as the dispersing agent to 1 part insecticides. After definite time intervals, the leaves were homogenized in an all-glass Potter-Elvehjem homogenizer, using 1 ml. of water for each 0.1 gm. of leaf material. The aqueous portion of the homogenate was then separated by centrifugation, and the metabolic products were extracted into an equal volume of chloroform. The chloroform solution was concentrated almost to dryness under vacuum, and the residue was chromatographed. These procedures assured that the leaves contained only translocated materials and were not contaminated from the direct application of the starting materials.

Table 1.—Oxidative metabolites of dithio-Systox and Thimet showing R_f values on propylene-glycol paper and I_{50} values for fly-head cholinesterase inhibition.

	R_f	Fly Head ChE I_{50} M.
dithio-Systox Series		
I. $(C_2H_5O)_2P(S)SCH_2CH_2SC_2H_5$	0.99	$>1\times10^{-4}$
II. $(C_2H_5O)_2P(S)SCH_2CH_2S(O)C_2H_5$	0.18	7×10^{-5}
III. $(C_2H_5O)_2P(S)SCH_2CH_2C(O)C_2H_5$	0.64	3.5×10^{-6}
IV. $(C_2H_5O)_2P(O)SCH_2CH_2SC_2H_5$	0.80	3.5×10^{-6}
V. $(C_2H_5O)_2P(O)SCH_2CH_2S(O)C_2H_5$	0.00–0.04	1.5×10^{-6}
VI. $(C_2H_5O)_2P(O)SCH_2CH_2S(O)C_2H_5$	0.03–0.08	6.0×10^{-7}
Thimet Series		
VII. $(C_2H_5O)_2P(S)SCH_2SC_2H_5$	0.99	2.5×10^{-5}
VIII. $(C_2H_5O)_2P(S)SCH_2S(O)C_2H_5$	0.31	3.7×10^{-6}
IX. $(C_2H_5O)_2P(S)SCH_2SC(O)C_2H$	0.66	4×10^{-8} [a]
X. $(C_2H_5O)_2P(O)SCH_2SC_2H_5$	0.53	5×10^{-7}
XI. $(C_2H_5O)_2P(O)SCH_2S(O)C_2H_5$	0.0	4×10^{-7}
XII. $(C_2H_5O)_2P(O)SCH_2SC(O)C_2H_5$	0.05	1×10^{-7}

[a] This sample is of questionable purity (see experimental section).

The technics for the measurement of radioactivity, for bioassay, and for cholinesterase determinations have been previously described (Fukuto *et al.* 1955).

ABSORPTION AND TRANSLOCATION.—The rates of absorption and translocation of dithio-Systox and of Thimet

were measured as has been done previously with the Systox isomers (Metcalf *et al.* 1955) by the topical application of 5 to 25λ volumes of the purified materials to the bases of the stems of cotton and lemon plants, followed by measurement of the chloroform- and water-partitioning radioactivity in the leaves at intervals after application, using three replicate plants for each material. Typical data are presented in figure 1, where a comparison is made of Systox thiol isomer, Thimet, and dithio-Systox applied to cotton plants.

There is little difference in the rate of uptake or decomposition of Thimet and dithio-Systox but Systox thiol isomer is initially absorbed and translocated at a rate about 1.5 to 2 times as great. This difference seems explainable by the comparative water solubilities of the systemics, which were determined by shaking P^{32}-radiotracers in large volumes of distilled water, filtering, and counting aliquots of the aqueous portions. The results obtained were: Systox thiono isomer 1250 p.p.m.; Systox thiol isomer 3900 p.p.m.; Thimet 85 p.p.m.; and dithio-Systox 66 p.p.m.

It will be noted from figure 1 that although Systox thiol isomer was initially absorbed and translocated more rapidly than Thimet or dithio-Systox, the relative rates of absorption and translocation of the last two materials increased as the experiment proceeded. Probably this increased rate of translocation results from the formation of the more water-soluble oxidative metabolites in the subcuticular layers of plant tissue around the region of application.

PLANT METABOLISM OF DITHIO-SYSTOX.—In view of the close structural similarity between the dithio-Systox and Systox isomers, it seemed probable that the dithio-compound could be metabolized in plants in an analogous manner, i.e., by oxidation to sulfoxide and sulfone derivatives. Cotton leaves were treated topically at the base of the stem with 25λ of P^{32}-dithio-Systox. The upper leaves were removed at intervals and homogenized in three times their weight of water, and the radioactivity was then partitioned between chloroform and water. Aliquots of the chloroform-partitioning fraction were concentrated to dryness, taken up in a small amount of acetone, and chromatographed on the propylene-glycol-impregnated paper. Within a few hours after application, four distinct spots were found on the papers, as shown in figure 2. The same sequence of spots was found after application to bean, lemon, and alfalfa plants. The typical R_f values for the chloroform-soluble metabolites from these four plant species are given in table 2, for comparison with the oxidative products from bromine-water oxidation (Fallscheer & Cook 1956). It is of interest that only minute traces of dithio-Systox R_f 0.95 to 0.99 were found in any of the plant experiments and that generally none of this compound was present. When the R_f values for the chloroform-soluble plant metabolites are compared with those for the known oxidation products (table 1), it will be seen that within the R_f reproducibility, the same sequence occurs, thus identifying the spots in table 2 and figure 2 as indicated by the compound number (see table 1).

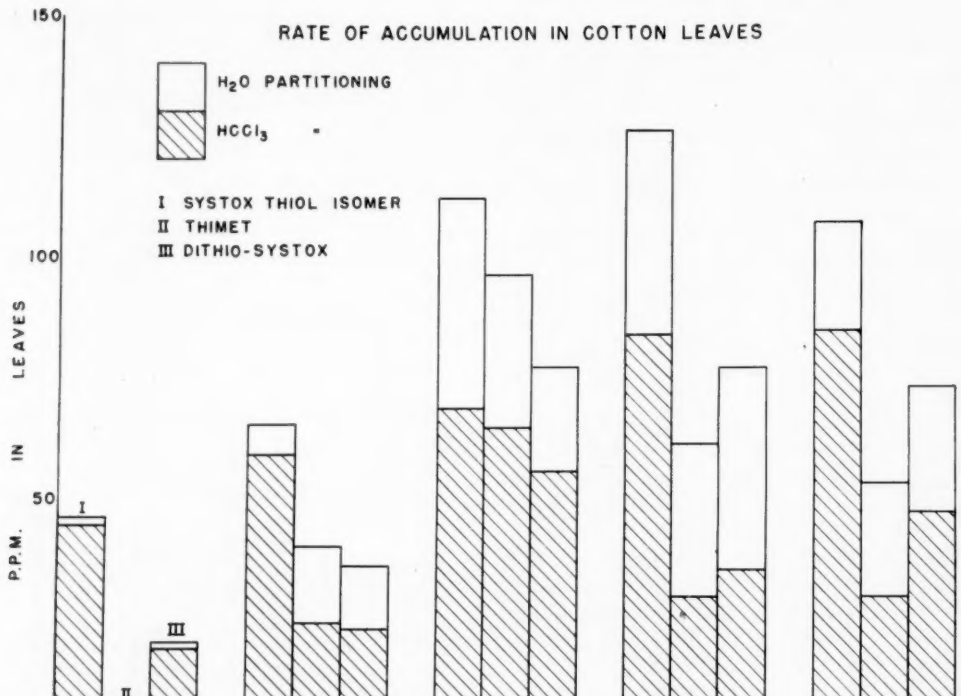

FIG. 1.—Rate of accumulation of metabolites of Systox thiol isomer, dithio-Systox, and Thimet in cotton leaves, following topical application of 5λ to base of stem.

Table 2.—R_f values on propylene-glycol paper for plant metabolites and oxidative products of dithio-Systox.

Compound (Table 1)	Chloroform-Soluble Metabolites After (Days)							Bromine-Water Oxidation After 30 Minutes
	Cotton		Alfalfa		Lemon		Bean	
	3	7	7	14	7	17	6	
V	0.0	0.0	0.02	0.05	0.0	0.0	0.026	0.0
VI	.09	.07	.07	.09	.07	.08	.07	.06
II	.29	.30	.27	.31	.29	.29	.31	.26
III	.69	.65	.63	.61	.59	.69	.53	.65

From inspection of a series of chromatograms of the radioactive chloroform-soluble plant metabolites produced over a period of several weeks, it was evident that the oxidative mechanism was proceeding in an orderly and measurable sequence of consecutive reactions. In order to evaluate these as cleanly as possible without interference from the gradual accumulation of translocated material, mature cotton leaves on long petioles were placed in 1 ml. of water containing known quantities of P^{32} dithio-Systox in colloidal suspension from a mixture of 3 parts Thiosolve emulsifying and dispersing agent and 1 part compound. The uptake of the entire volume of solution was quantitative within 30 minutes to 1 hour. The leaves were than removed to stand in fresh water, and remained in a green and healthy condition for periods up to several weeks. At appropriate intervals after treatment, pieces of leaf were removed, homogenized, and partitioned between water and chloroform; the chloroform-soluble fraction was then chromatographed. From the chromatograms, as shown in figure 2, the relative amounts of each compound present were determined by serial radioactive counting. The data were plotted as shown in figure 3, according to the standard treatment for consecutive reactions (Glasstone 1940), evaluating the amount of each compound as a fraction of the total radioactivity in the leaf.

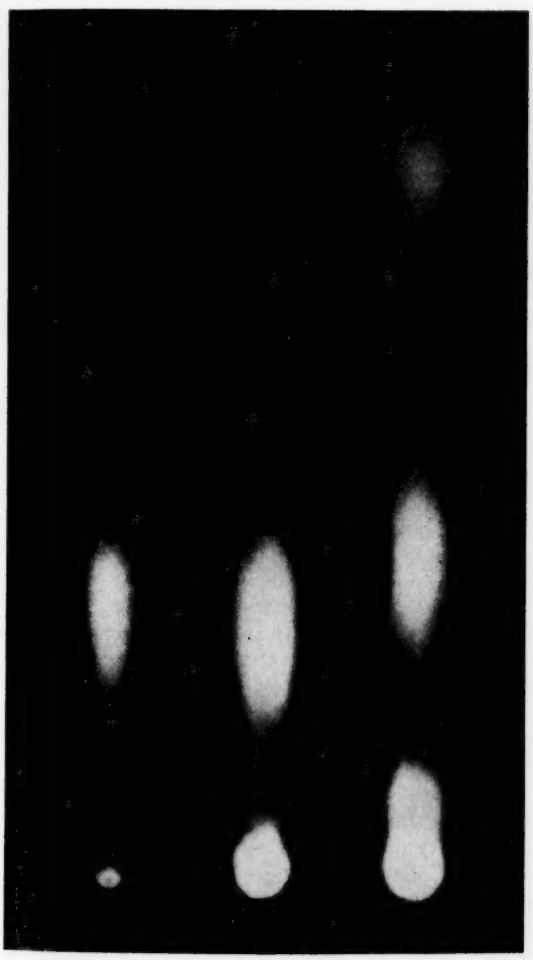

FIG. 2.—Chromatograms of chloroform-soluble radioactive metabolites from cotton leaves treated with translocated P^{32}-dithio-Systox: 5 hours (left), 75 hours (center), and 168 hours (right) after treatment. The spots, in ascending order, represent Systox thiol isomer sulfoxide (V), Systox thiol isomer sulfone (VI), dithio-Systox sulfoxide (II), and dithio-Systox sulfone (III).

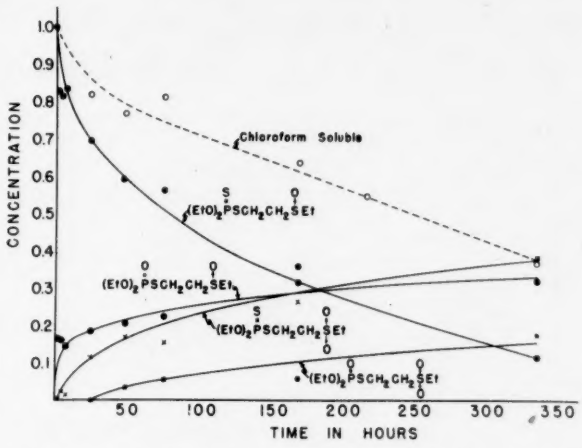

FIG. 3.—Graph showing relative amounts of dithio-Systox metabolites as fractions of total chloroform-partitioning P^{32} in extracts of isolated cotton leaves at various intervals after treatment. The values for the chloroform-soluble material represent the fraction of total P^{32} which partitioned into chloroform.

Figure 3 shows quite clearly the consecutive first-order reactions in the living plant environment resulting from the oxidative metabolism of dithio-Systox. These may be expressed as shown in figure 4. Only minute traces of dithio-Systox (I) were ever found in the leaves, even at intervals as short as 1 hour after treatment. Thus k_1 is immeasurably fast, as has been reported for the persulfate oxidation of sulfides to sulfoxides (Howard & Levitt 1953). The rate of oxidation of the sulfoxide (II), as measured by its disappearance, proceeds in an exponential manner, with the rate given by:

$$-\frac{d[II]}{dt} = [II](k_2+k_3)$$

A graphic solution of this equation gave a value of $k_2+k_3=0.0070$ hr.$^{-1}$.

The rates of formation of compounds III and V from II, and of VI from III and V, represent highly complicated differential equations whose solution has not yet been accomplished.

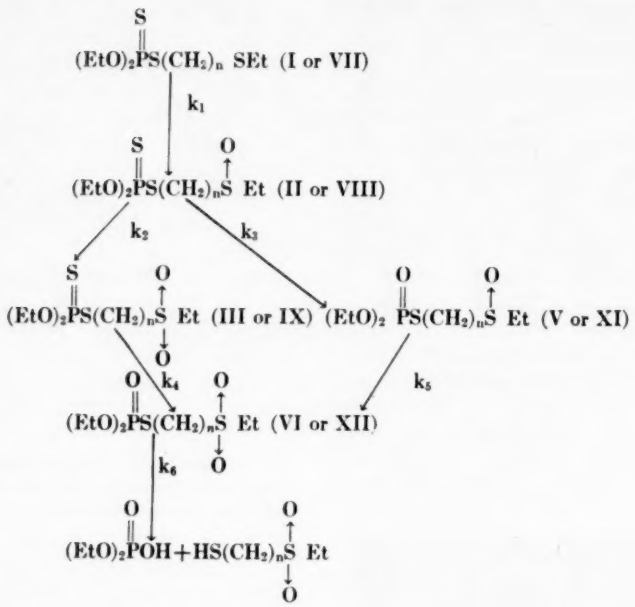

FIG. 4.—Sequence of consecutive reactions in cotton leaf from application of dithio-Systox or Thimet (n=1 for Thimet; n=2 for dithio-Systox.)

The rate of loss of total chloroform-soluble material in the plant, as plotted in figure 3, presumably represents hydrolysis of the four metabolites II, III, V, and VI to form inactive diethylphosphoric acids. Compound VI should theoretically be the final oxidative metabolite and should hydrolyze most readily, as has been depicted in figure 4. This also closely approximated a first-order reaction with a value of $k=0.0031$ hr.$^{-1}$.

From the measurements made on other plants (table 2), it appears that this information represents a fairly typical picture of the sequence and rates of reaction of the dithio-Systox metabolites in plants. Although the rates of reaction may be expected to vary slightly from plant species to plant species and according to the stage of growth, this information may be used as a guide to the relative proportions of the metabolites present at intervals after application. It is obvious that the oxidative metabolites *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate (V), and *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate (VI) are the identical compounds which are the final oxidative plant metabolites of Systox thiol isomer (Fukuto *et al.* 1955, 1956). Thus assessment of the possible toxic hazards of residues resulting from treatment with dithio-Systox can be supplemented by the much more extensive prior work carried out with Systox thiol isomer.

These data confirm a point which has previously been obscure regarding the plant metabolism of phosphorothionates. It appears from figure 3 that the P=S group is oxidizable *in vivo* at a rate which is comparable to that of the oxidation of S→O to O←S→O. From this evidence, therefore, it seems fairly certain that the Systox thiono isomer must be oxidized in plants to both *O,O*-diethyl *O*-ethyl-2-sulfinylethyl phosphate and *O,O*-diethyl *O*-ethyl-2-sulfonylethyl phosphorothionate. The former compound is so highly unstable, however, that it does not persist very long, and this has undoubtedly rendered its detection difficult. Because the sulfide sulfur is much more readily oxidized to S→O than P=S is oxidized to P=O, it seems clear that compound (IV) Systox thiol isomer should not be formed in appreciable amounts in plants from dithio-Systox, nor has its presence been detected.

PLANT METABOLISM OF THIMET.—The behavior of Thimet in the living plant environment is very similar to that of dithio-Systox. The same technics described for the latter compound were used to show that four distinct plant metabolites of Thimet were formed within a few hours after uptake of the compound. When separated by paper chromatography, these metabolites had the R_f values shown in table 3. As with the dithio-Systox series, the plant oxidation products of Thimet were formed in the same sequence as with bromine-water oxidation and corresponded in R_f values to the known oxidative metabolites shown in table 1.

As with dithio-Systox the rate of appearance of the spots (which were almost identical with those shown in the chromatograms of figure 2) from the Thimet metabolites indicated a series of consecutive reactions which were evaluated as described above and are plotted in figure 5. The consecutive reactions (figure 4) are quite evidently analogous to those with dithio-Systox but certain minor differences exist. The initial oxidation of Thimet in the plant was less rapid than the oxidation of dithio-Systox, and traces of Thimet were found up to 3 days, although these never exceeded 5% of the total radioactivity. It also appeared that the rate of oxidation of the two sulfoxides (VIII) and (IX) was measurably slower in the Thimet series than in the dithio-Systox series. This is explainable, since in the Thimet series the closer proximity of the electrophilic phosphorus atom to the sulfide sulfur may be expected to decrease the electron

Table 3.—R_f values on propylene-glycol paper for plant metabolites and oxidative products of Thimet.

Compound (Table 1)	Chloroform-Soluble Metabolites After (Days)					Bromine-Water Oxidation After 30 Minutes
	Cotton		Alfalfa		Lemon	
	3	7	5	7	17	
XI	0.017	0.0	0.0	0.0	0.0	0.0
XII	.08	.07	—	.06	.10	.05
VIII	.38	.26	.23	.35	.43	.36
IX	.68	.57	.53	.66	.74	.67

density around the sulfur atom by a $-I$ effect and thus to decrease its reactivity. Thus with the Thimet metabolites the consecutive first-order reactions are shown in figure 4, and the values for k_1, k_2 and k_5 are measurably slower. Of the readily calculable values, the sum of k_2+k_3 for the Thimet series was almost exactly half that for the dithio-Systox series or 0.0035 hr.$^{-1}$ vs. 0.0070 hr.$^{-1}$. Casida *et al.* (1956) have suggested the same oxidative metabolites following Thimet treatment of cotton plants.

ANTICHOLINESTERASE ACTIVITY OF PLANT METABOLITES.—The relative toxicities of the oxidative products of dithio-Systox and Thimet as expressed by the I_{50} values for fly-brain cholinesterase inhibition (table 1) are of interest from the standpoint of theoretical chemistry. It is evident that replacement of P=S with P=O markedly increases the activity by increasing the instability of the molecule. The phosphorus is considerably more electrophilic in the P=O compounds (Fukuto 1957), and this weakens the P—S-ester bond, thus facilitating hydrolysis and accelerating phosphorylation of the enzyme. The successive oxidation of the sulfide sulfur to sulfoxide and to sulfone increases the electron-withdrawing power of this portion of the molecule, thus increasing the instability of the compound at the P—S-ester bond, and promoting phosphorylating ability. The Thimet series with the single methylene groups between the two sulfur atoms is somewhat more active than the dithio-Systox series, in which an ethylene group is present between the two sulfurs. This is to be expected, since the —I effect of the electrophilic phosphorus and of the sulfoxide and sulfone groups decreases with the increased length of the aliphatic chain.

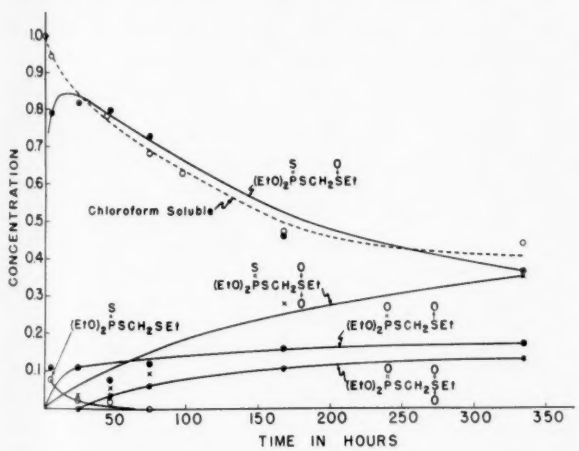

FIG. 5.—Graph showing relative amounts of Thimet metabolites as fractions of total chloroform-partitioning P^{32} in extracts of isolated cotton leaves at various intervals after treatment. The values for the chloroform-soluble material represent the fraction of total P^{32} which partitioned into chloroform.

Table 4.—Residues of dithio-Systox and metabolites in cotton plant parts and extractions 52 days after mature plants were sprayed with dithio-Systox at the rate of 1 pound per acre in 25 gallons of water from a 25% emulsion concentration.

Plant Parts and Extractions	Parts per Million dithio-Systox
Leaves (initial)	66.0
Green leaves at harvest	
Water phase	0.8
Chloroform phase	0.99
Yellow leaves at harvest	
Water phase	28.3
Chloroform phase	2.5
Mature seeds (ground, 200 mesh)	
Total	28.4
Chloroform-extracted	0.51
Water-extracted	11.5
Green seeds	
Total	2.74
Chloroform-extracted	0.08
Water-extracted	1.80
Extracted oil	
Chloroform-extracted	3.02
Skellysolve B-extracted	1.24
Phospholipids	2.89[a]
Toxic phosphorus esters	0.133[a]
Cake	
Chloroform-extracted	26.7
Fiber	1.35

[a] Dr. Helmut Tietz kindly fractionated the extracted oil by acetone-$MgCl_2$ precipitation of phospholipids (Mühlmann & Tietz 1956) and obtained 72.3% of the total P^{32} in the precipitate and 23.3% of the total P^{32} in a subsequent methanol extract. Thus, it must be concluded that only the unextractable and non-precipitable P^{32}, or 4.4% of the total, is in the form of toxic phosphorus esters, equivalent to not more than 0.133 p.p.m.

RESIDUES IN TREATED PLANTS.—The P^{32}-tracers of the compounds described in this paper are particularly applicable to determinations of the nature and magnitude of plant residues resulting from treatment with these systemics. The use of these tracers in the evaluation of seed treatments will be described in a subsequent paper, but two typical studies are reported here.

Table 4 shows the results of spraying mature cotton plants with dithio-Systox at 1 pound per acre, 52 days before harvest. The seeds contained relatively high amounts of P^{32}, of which only about 10% was extractable from chloroform. The resulting crude-oil fraction containing 3.02 p.p.m. P^{32} as dithio-Systox was further fractionated by using the method of Mühlmann & Tietz (1956) and proved to contain 95.6% of the total P^{32} as acetone-$MgCl_2$-precipitable phospholipids. These must form from degradation of the P^{32} esters to orthophosphates, which are then resynthesized in the plant. The 4.4% remainder of the total P^{32} that was unaccounted for

must be assumed to be toxic phosphorus esters and thus represents a maximum of 0.133 p.p.m. in the oil.

The data in table 5 was obtained from a 200 sq. ft. field plot of alfalfa plants grown from seed treated with P^{32}-dithio-Systox at the rate of 1 pound (as a 50% charcoal powder) to 25 pounds of seed applied as a slurry of 8% water (by weight of seed) with 2. oz of sticker.[3] The P^{32} was present in large amounts in the trifoliate leaves of the plants, 2 weeks after the seeds were planted and was largely in the chloroform-soluble fraction. However, as the plants rapidly increased in size, both the total P^{32} and the chloroform-soluble portion decreased, so that after 8 weeks, or about the minimum time for a first cutting there were no toxic metabolites detectable by cholinesterase assay, and only 0.007 p.p.m. by radioassay.

From inspection of a series of chromatograms of the radioactive chloroform-soluble metabolites as found in the leaves of the harvested alfalfa plants, it was possible to plot a series of curves as had been done with the isolated cotton leaves noted above. After 4 weeks the amounts of chloroform-soluble metabolites were too small for extraction and preparation of satisfactory chromatograms. The results are shown in figure 6. The data for 7 days are from extracts of the cotyledons and show the presence of some dithio-Systox; none however, was found in any of the later extracts of the trifoliate leaves. This somewhat slower transformation of dithio-Systox to oxidative metabolites may in part be due to the continuing supply of dithio-Systox from the seed treatment. The results from the seed-treated field-grown alfalfa plants show very good agreement with the results from the laboratory-treated cotton leaves, although the rates of metabolism are somewhat different. In the field alfalfa, oxidation of the thioether moiety to the sulfoxide was very rapid just as it was in the cotton but oxidation of S→O to O←S→O occurred more rapidly than oxidation of P=S to P=O. In the cotton leaves these reactions were approximately equal. Thus the final toxic metabolites in the alfalfa are *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorodithioate (III) and *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate (VI). After 3 weeks it is apparent that compound III had reached its maximum

Table 5.—Amounts of metabolites in trifoliate leaves of alfalfa plants grown from seed treated with P^{32}-dithio-Systox at 1 pound (as 50% charcoal powder) per 25 pounds of seed, with 2 ounces sticker.[a]

Weeks After Treatment	Plant Weight (Mg.)	Metabolites in γ per Gm. Fresh Weight of Leaves (Calculated as Dithio-Systox)			
		Total P^{32}	$HCCl_3$ Fraction	H_2O Fraction	ChE Assay
2	57	180.3	121.5	67.8	
3	237	51.6	22.0	29.6	
4	400	37.6	12.1	25.5	
5	750	13.3	1.14	12.2	
6	1300	5.2	0.50	4.7	
7	3700	2.38	0.21	2.17	
8	—	2.57	0.007	2.56	0.0[b]

[a] Colloidal Seed Coat, Colloidal Products, Co.

[b] Calculated as $(Et+O)_2PSCH_2CH_2SC_2H_5$ (O and O)

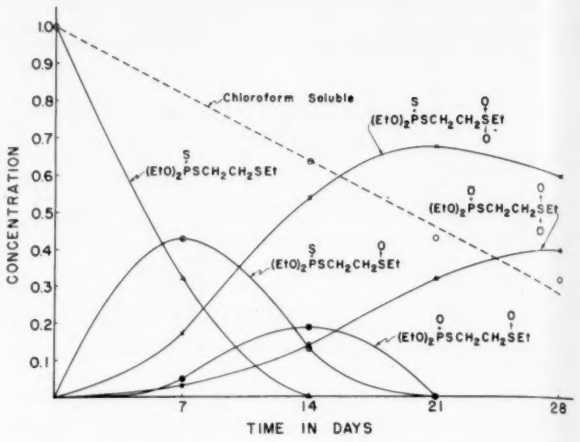

FIG. 6.—Graph showing relative amounts of dithio-Systox metabolites as fractions of total chloroform-partitioning P^{32} in extracts of alfalfa leaves at various intervals after seed treatment with 50% charcoal powder at 1 pound to 25 pounds of seed.

concentration and was being converted to VI. It must be remembered that the toxic metabolites were concurrently being hydrolyzed to non-toxic phosphoric or thiophosphoric acids as shown by the decline in chloroform soluble metabolites in the leaf extracts (see figure 6 and table 5). At 8 weeks there were essentially no toxic metabolites remaining in the alfalfa, 0.007γ/gm. maximum by radioassay and none detectable by cholinesterase assay.

CHOLINESTERASE ASSAY OF PLANT RESIDUES OF DITHIO-SYSTOX AND THIMET.—The cholinesterase assay using human blood plasma and the electrometric technic as developed by Hensel *et al.* (1954) for Systox, is applicable to the detection of residues of dithio-Systox and Thimet. It is clear that the presence of the oxidative metabolites demonstrated in this paper has a pronounced influence upon the interpretation of data from this analytical method.

The ultimate toxic metabolites present in harvesttime residues are dependent both upon the interval between application and harvest and upon the method of application. The seed treatments in particular provide for the continuous absorption of toxicant over a comparatively long period of time as compared with spray treatments. Hence the decomposition rates (figure 6) are skewed as compared with those where the toxicant is absorbed into the plant over a very short period of time (figure 3). With dithio-Systox the harvesttime residues may contain a mixture of varying amounts of *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate or Systox thiol isomer sulfoxide (V); *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate, or Systox thiol isomer sulfone (VI); and *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorodithioate (III). The first two named are the principal toxic metabolites of Systox thiol isomer and the applicability of the cholinesterase assay to these metabolites has been discussed by Metcalf *et al.* (1955). The I_{50} values for human plasma cholinesterase and the approximate limits of detection of these compounds in aqueous homogenates are

[3] Colloidal Seed Coat, Colloidal Products, Co.

Table 6.—Inhibition of human plasma cholinesterase and the limits of detection of electrometric technic for oxidative metabolites of dithio-Systox and Thimet.

COMPOUND	I_{50} PLASMA CHOLINESTERASE γ/ML.	LIMIT DETECTION AT 20% INHIBITION γ/ML
O,O-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate (V)	0.75	0.3
O,O-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate (VI)	0.25	0.1
O,O-diethyl *S*-ethyl-sulfinylmethyl phosphorothiolate (XI)	0.6	0.2
O,O-diethyl *S*-ethyl-sulfonylmethyl phosphorothiolate (XII)	0.065	0.03

given in table 6. It is suggested that compound VI be employed as the the standard for the cholinesterase determination of dithio-Systox metabolites since it is the most active inhibitor thus giving the maximum sensitivity, and is logically the final product in the series of oxidative transformations.

With Thimet, exactly parallel considerations apply and the harvesttime residues will consist of a mixture of *O,O*-diethyl *S*-ethylsulfinylmethyl phosphorothiolate (XI); *O,O*-diethyl *S*-ethylsulfonylmethyl phosphorothiolate (XII); and *O,O*-diethyl *S*-ethylsulfonylmethyl phosphorodithioate (IX). The I_{50} values for human plasma cholinesterase and the approximate limits of detection of compounds XI and XII are given in table 6. From the considerations mentioned above it is suggested that compound XII be employed as the standard in the cholinesterase determination of Thimet metabolites.

SUMMARY AND CONCLUSIONS.—The plant metabolism of dithio-Systox and of Thimet was investigated by the use of P^{32}-radiotracers. It was demonstrated that these two systemic compounds were initially absorbed and translocated into cotton and lemon plants at approximately equal rates, but only from about 0.5 to 0.75 times as fast as Systox thiol isomer.

The predominant processes of plant metabolism for both dithio-Systox and Thimet are primarily oxidative. Following uptake of dithio-Systox by cotton, lemon, bean, or alfalfa plants, the mercaptosulfur is oxidized very rapidly to produce the sulfoxide *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorodithioate and more slowly to produce the corresponding sulfone, *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorodithioate. Both the sulfoxide and the sulfone are also oxidized at the thionosulfur to produce *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate and *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate. Thus at intervals of a few days to a month after application, all four compounds may be present in plant residues, but no dithio-Systox is found. The sequence of consecutive reactions for dithio-Systox and for Thimet in isolated cotton leaves has been plotted, and a beginning has been made in establishing the kinetics of these. Thimet behaves in a manner completely analogous to that of dithio-Systox but exhibits somewhat different rates of metabolism.

Typical harvest residues of dithio-Systox metabolites are presented from experiments using this material as a direct spray for cotton plants and as a seed treatment for alfalfa. The ultimate toxic residues are present in fractional parts per million. The application of the knowledge of plant metabolism of these compounds to analytical studies by the cholinesterase method is also discussed.

All the simple oxidative metabolites of dithio-Systox and Thimet have been prepared in the pure state, and their properties are recorded.

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The Systemic Action of Lindane in Alfalfa upon the Meadow Spittlebug[1]

C. S. Koehler and George G. Gyrisco, *Cornell University, Ithaca, N. Y.*

Systemic action of various phosphorus-containing compounds has long been recognized (Schrader 1947, Bennett 1949) and widely demonstrated by many recent workers. Little attention, however, has been paid to the chlorinated hydrocarbons in this regard. Frequent reports in the literature (Snapp 1947, Stoker 1948, Greenwood & Tice 1949) and among entomologists concerning off-flavors in crops which have been treated with BHC have probably been the strongest evidence of the absorption of substances into treated plants. While many of these objectionable flavors were eliminated with the purification of the material into lindane, it has not been possible to eliminate them entirely.

Howe (1950) noted a large reduction in the number of squash vine borer, *Melittia cucurbitae* (Harr.), larvae attacking squash grown in soil treated with BHC at a rate of 2 pounds per acre of the gamma isomer. Surprisingly, the following year similar results were observed upon the same plot with no additional treatment. No provision, unfortunately, had been made to assess possible fumigant action. Starnes (1950) found significant reductions in uncaged populations of the rose aphid, *Macrosiphum rosae* (L.), on potatoes grown in the greenhouse in soil treated with lindane at rates of 10, 20, and 40 pounds per acre. Although no mention was made which would indicate that fumigant action had been controlled in these tests, bioassay of potato vines indicated the presence of a toxic material. Therefore, under conditions which eliminated the possibility of fumigant and contact action, experiments were initiated in 1954 to determine whether alfalfa plants treated with foliar or soil applications of lindane exerted a systemic effect upon the nymph of the meadow spittlebug, *Philaenus leucophthalmus* (L.), and if so, to investigate some of the aspects of this phenomenon.

General Procedure.—Alfalfa plants grown from cuttings were potted in metal quart containers and treated with lindane as both soil and foliar applications. Each treatment was replicated four times and all experiments were laid out in randomized complete block designs.

All plants were infested with nymphs of the meadow spittlebug, field-collected and sorted as to the same approximate age and size. Nymphal mortality counts made at intervals after treatments were used as a criterion of systemic action. All data were analyzed statistically at the 5% level, using the multiple range test as suggested by Duncan (1953).

Methods and Results.—*Soil Application.*—After diluting emulsifiable lindane to make up concentrations of 0.25, 0.10, and 0.05% actual material, an arbitrary quantity of 20 ml. of the emulsion was poured directly onto the soil surrounding the stem of each plant. In order to prevent any possible toxic fumes from acting upon the test insects, each pot was capped immediately after treatment with a cellulose acetate disc which allowed the foliage to protrude above the cap. All other means of vapor escape were eliminated by sealing the disc firmly to the pot with drafting tape and caulking the space which remained between the plant stem and the centrally punched hole in the disc with modeling clay.

Six days after soil treatment, 10 spittlebug nymphs were transferred onto the foliage of each plant. Counts were made of the nymphs remaining alive 20 and 50 hours later.

Between the time of application to the soil and the time the first counts were taken, sufficient toxic material had been translocated to the terminal portions of the plants to cause significant differences in nymphal mortality on the treated and untreated plants (table 1). After 50 hours no differences in nymphal mortality existed among plants treated with any of the concentrations of lindane used, although significant differences in nymphal mortality continued to be maintained between all treatments and the check.

To determine the reliability of the described method of capping pots, stems of untreated plants were bent over and affixed in such a manner that their foliage was in proximity to adjacent plants whose soil had been treated with 0.25% lindane and whose pots had been capped. Any toxic vapors evolving from around the caps of the treated plants would therefore affect test insects transferred onto both the treated and untreated plants. If vapors were not escaping from the soil of the capped plants, nymphs would be killed only upon those plants whose roots were in contact with the lindane. Test insects were prevented from moving between the foliage of treated and untreated plants.

It was found that a significant difference in spittlebug nymph mortality existed between treated capped plants and untreated plants whose foliage was in proximity to the treated plants. It was therefore concluded that the cellulose acetate disc method of capping pots could be completely relied upon to prevent fumigant action from interfering with systemic action.

From time to time during the period in which spittlebug nymphs were available in the field, plants which had been treated with concentrations of 0.25, 0.10, or 0.05% lindane were reinfested with test insects in order to determine the length of time systemic action would persist following an initial soil application. Fifty hours after each reinfestation, counts were made of the surviving nymphs on each plant.

During the entire 6-weeks period following soil treatment, significant differences in nymphal mortality were maintained between all treated plants and the untreated check lot (table 2). Systemic action may even have persisted longer than 6 weeks; the formation of some callow adults within spittle masses on the test plants and the degenerating condition of the plants made it necessary to discontinue this phase of the study at that time.

Foliar Application.—Plants, each consisting of two long stems were placed individually into a slotted cardboard carton so that it was possible to spray only one

[1] Some of these data were presented by the senior author at the Seventeenth Annual Insecticide and Fungicide Conference held at Ithaca, New York, November 8–10, 1955. Accepted for publication January 8, 1957.

Table 1.—Mortality of spittlebug nymphs[a] that were transferred to alfalfa plants 6 days after soil treatment with lindane emulsions.

No. Hours After Transfer	Average Percentage Nymphal Mortality[b]			
	Percentage Active Toxicant of Lindane Emulsion			
	0.25	0.1	0.05	0.0
20	100.0	95.0	77.5	12.5
50	100.0	100.0	95.0	20.0

[a] Ten nymphs transferred to each plant.
[b] For analysis, percentage mortality data transformed into angles = arc sin $\sqrt{\text{percentage}}$. Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

stem of each plant. These were sprayed until run-off occurred with concentrations of lindane emulsion, similar to those used previously, with a small hand atomizer.

After 5 days the treated stem of each plant was removed and test insects were transferred onto the remaining untreated one. At various intervals thereafter, nymphal mortality counts were made on all plants.

At the 18-hour interval, significant differences in insect mortality existed between plants which had been sprayed with either 0.25% or 0.10% lindane as compared with the check plants (table 3). Similar results were obtained after 30 hours.

DISCUSSION.—If lindane is capable of acting as a systemic insecticide in the laboratory, it is feasible that such action also takes place to some extent in the field. While early applications in the field probably act first in a contact or fumigant capacity, the systemic action of lindane may account in part for the high specificity of that material in spittlebug nymph control. Furthermore, the extreme effectiveness of low gallonage, weed sprayer application of lindane may be associated with the systemic capabilities of that insecticide. Although applications of this type often result in somewhat incomplete coverage of the plants, control of less than 100% of the nymphs is usually the exception.

SUMMARY.—The systemic action of lindane was demonstrated qualitatively by transferring field-collected meadow spittlebug nymphs onto treated alfalfa plants and recording nymphal mortality at intervals thereafter. It was shown that plants whose soil had been treated with an initial application of 20 ml. of 0.25, 0.10, or 0.05% lindane emulsion were capable of exerting a toxic influence for a period of at least 6 weeks. These experiments were conducted under conditions which eliminated the possibility of fumigant and contact action interfering with systemic action.

The ability of a toxic material to be translocated after a foliar application of lindane was demonstrated by spraying a portion of the plant, removing the treated part 5 days later, and allowing test insects to feed upon the untreated foliage. It was found that systemic action was considerably more powerful within plants whose soil had been treated than within plants receiving foliar applications of comparable concentrations.

These results suggest that the systemic action of lindane may account in part for the high specificity of that insecticide for spittlebug nymph control and may be responsible for the good success of weed sprayer applications of small amounts of lindane sprays.

Table 2.—Percentage mortality of spittlebug nymphs[a] 50 hours after reinfestation of alfalfa plants grown in soil that had been treated with lindane.

No. Days After Treatment	Average Percentage Nymphal Mortality[b]			
	Percentage Active Toxicant of Lindane Emulsion			
	0.25	0.1	0.05	0.0
6	100.0	100.0	95.0	20.0
14	95.0	77.5	82.5	7.5
25	97.5	100.0	80.0	22.5
41	97.5	87.5	87.5	42.5

[a,b] Same as for table 1.

Table 3.—Percentage mortality of spittlebug nymphs[a] transferred to unsprayed parts of alfalfa plants after one-half the foliage had been sprayed with lindane emulsion until run-off occurred and removed 5 days later.

No. Hours After Transfer	Average Percentage Nymphal Mortality[b]			
	Percentage Active Toxicant of Lindane Emulsion			
	0.25	0.1	0.05	0.0
18	30.0	20.0	10.0	2.5
30	37.5	35.0	17.5	5.0

[a,b] Same as for table 1.

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Excretion of Endrin in the Milk of Cows Fed Endrin-Sprayed Alfalfa and Technical Endrin[1]

R. E. Ely[2] and L. A. Moore, *Dairy Husbandry Research Division*
and
R. H. Carter and B. A. App, *Entomology Research Division. Agric. Res. Serv. U.S.D.A.*

Endrin[3] is effective in controlling a wide range of insects and is promising for use on many important forage crops that are fed to livestock.

The acute oral toxicity of endrin to laboratory animals has been studied (Sherman & Rosenberg 1954, Treon *et al.* 1955), and considerable variation in the susceptibility of several species to orally administered endrin has been reported (Treon *et al.* 1955). Female rats and guinea pigs were more susceptible to endrin toxicity than males of either species (Treon *et al.* 1955).

Information is needed on the endrin residues on forage crops after spraying with recommended dosages and on the endrin content of animal products that may result from the feeding of endrin-sprayed forage to livestock. No reports are available on the excretion of endrin in the milk of dairy cattle.

The object of this investigation was (*a*) to determine if endrin is excreted in the milk of cows fed hay made from forage that had been sprayed with endrin approximately 1 week before havesting and (*b*) to study the effect of feeding various intakes of endrin in soybean oil to dairy cows on the concentration of endrin in the milk.

Experimental Procedure.—Two plots of second growth alfalfa were sprayed with 2.7 oz. and 6.6 oz. of endrin per acre, respectively, in 1953. One plot of second growth alfalfa was sprayed with 7.8 oz. of endrin per acre in 1954. All plots were harvested 1 week after spraying and the alfalfa was field-cured, field-baled, and stored approximately 6 months in a hay barn. Each lot was then fed to four normal, healthy, milking cows as their entire roughage. The hay harvested in 1953 was fed for 48 days and that harvested in 1954 was fed for 63 days. All animals received sufficient hay to satisfy their appetites and provide a 10 to 15% roughage refusal. A milking herd grain mixture was also fed, the amount per cow depending on the level of milk production. Daily records were kept of the amounts of feed fed and refused and the amounts of milk produced.

Samples of hay were taken from several locations in each bale fed and composited for analyses for each10-day feeding period. An aliquot of the milk produced by each animal for two consecutive days during each 10-day period was used for endrin and butterfat analyses. Each animal was weighed on two successive days at 10-day intervals.

The endrin content of the hay was calculated from the organic chlorine content of the sprayed hay after correction for the organic chlorine content of the untreated hay. The organic chlorine content of the hay was determined by extraction of the hay with Skellysolve B, condensation of the extract and combustion by a modification of the method of Agazzi *et al.* (1953) followed by amperometric titration with silver nitrate.

Analyses of the milk samples were made on butterfat separated by the method of Sager & Sanders (1952). The fat was saponified with alcoholic KOH and the soap solution was extracted three times with Skellysolve B. The combined Skellysolve B extracts were washed with water, concentrated, and burned in a Shell type combustion apparatus by a modification of the method of Agazzi *et al.* (1953) followed by amperometric titration with 0.001 N silver nitrate. Details of the procedure have been presented previously by Ely *et al.* (1954).

After the period of feeding endrin-sprayed hay in 1953 the eight animals received a normal ration containing no insecticide residue and various levels of intake of endrin in soybean oil administered by capsule.

Results and Discussion.—A summary of the results of feeding the endrin-sprayed hay during the two seasons is presented in table 1.

Hay made from the alfalfa plots sprayed with 2.7, 6.6, and 7.8 oz. of endrin per acre had average endrin contents of 2.8, 3.7, and 1.9 p.p.m., respectively, at the time of feeding. Much greater losses occurred on the alfalfa after spraying and during the curing period in 1954 than in 1953. All animals consumed normal quantities of hay for their weight on ad libitum feeding.

The average endrin content of the milk of the four cows receiving the hay containing endrin residues of 1.9, 2.8, and 3.7 p.p.m. was <0.05, 0.14, and 0.15 p.p.m., respectively. Average daily intakes of the individual animals ranged from 0.04 to 0.11 mg. per kilogram of body weight. A daily intake of approximately 0.05 mg. per kilogram of bodyweight of endrin and below did not result in the excretion of measurable amounts of endrin in the milk. Average daily endrin intakes of from 0.06 to 0.11 mg. per kilogram of body weight gave average concentrations of endrin in the milk of approximately 0.1 to 0.2 p.p.m. Since these values are two to four times the standard deviation, they are considered significant.

The results of feeding various levels of endrin in soybean oil to milking cows are summarized in table 2. Average daily intakes of 40 to 100 mg. of endrin in soybean oil (0.11 to 0.24 mg. per kilogram of body weight) were below the dosage required for the excretion of measurable amounts of endrin in the milk. An average daily intake of 200 mg. (0.60 mg. per kilogram of body weight) resulted in the possible excretion of endrin in the milk and intakes of 500, 600, and 800 mg. of endrin daily (1.42, 1.67, and 2.33 mg. per kilogram of body weight, respectively) gave detectable amounts of endrin in the milk.

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[2] Present address: State Experiment Stations Division, U. S. Dept of Agriculture, Washington, D.C.

[3] Coined name for 1,2,3,4,10,10-hexachloro-6,7,-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*, *endo*-5, 8-dimethanonaphthalene.

Table 1.—Results of feeding endrin-sprayed hay to dairy cows at Beltsville, Md. 1953–1954.

Animal	Endrin per Acre (Oz.)	Average Daily Endrin Intake P.p.m.[a]	Intake Mg./Kg[b]	Intake Mg.	Output in Fat Corrected Milk P.p.m.	Output in Fat Corrected Milk Mg.
Cows fed for 63 days—1954						
N 186	7.8	1.58	0.04	21.3	<0.05	
N 635	7.8	1.54	.04	17.4	< .05	
N 645	7.8	1.46	.05	17.1	< .05	
N 656	7.8	1.62	.05	20.9	< .05	
Cows fed for 48 days—1953						
N 675	2.7	1.90	.06	20.5	.09± .03[c]	
N 668	2.7	1.93	.07	23.5	.14± .01	2.9
N 649	2.7	1.97	.08	34.6	.14± .02	2.8
N 684	2.7	2.08	.08	28.7	.21± .05	1.7
N 681	6.6	2.41	.06	23.6	.17± .04	1.1
N 666	6.6	2.58	.08	37.4	.11± .02	1.6
N 667	6.6	2.63	.09	33.4	.18± .10[d]	2.3
N 341	6.6	2.76	.11	43.9	.13± .01	2.2

[a] Endrin calculated as p.p.m. of the total feed consumed.
[b] Milligrams of endrin fed per kilogram of bodyweight.
[c,d] Bioassay, pomace flies, indicated 0.13 p.p.m. average for each.

Table 2.—Results of feeding technical endrin in soybean oil to dairy cows at Beltsville, Md. 1954.

Animal	Feeding Period (Days)	Endrin Fed Daily Mg.	P.p.m.[a]	Mg./ Kg.[b]	Average Daily FCM (Lb.)	Endrin in Fat Corrected Milk P.p.m.	Mg. Daily
N 341	56	40	2.5	0.11	26.9	<0.05	
N 649	64	50	2.9	0.11	35.8	<0.05	
N 666	56	60	4.0	0.14	21.4	<0.05	
N 667	56	100	7.3	0.24	23.2	<0.05[e]	
N 668	64	200	13.4	0.60	41.7	0.05	.8
N 681	2	400	50.5	1.11	15.9	0.20[d]	
N 684	14	400	39.0	1.17	22.0	0.35[e]	
N 675	64	500	40.2	1.42	34.9	0.25 ± .05[e]	3.7
N 684	4	600	58.2	1.77	25.5	0.20[f]	
N 681	56	200	75.8[g]	1.67[g]	15.8	0.15 −± .05	.9
N 684	22	800	77.7[h]	2.33[h]	23.1	0.27± .12	1.8

[a] Endrin calculated as p.p.m. of the total feed consumed.
[b] Milligrams of endrin fed per kilogram of body weight.
[c] Bioassay, pomace flies, film exposure indicated averages of 0.09 and 0.41 p.p.m. of endrin in the FCM for animals N 667 and N 675, respectively.
[d] One composite milk sample taken on last day of administration and first day after cessation. This period followed the 600 mg. daily intake shown below.
[e] Average value for two composite milk samples taken on the second and third and eleventh and twelfth days of administration.
[f] One composite milk sample taken on last 2 days of administration.
[g] Endrin dosage of 600 mg. daily was administered intermittently on 34 days of the 56-day period and withheld on 22 days to permit recovery of symptoms of severe toxicity. Average daily endrin intakes for the entire 56-day period are 45.5 p.p.m. of the ration and 1.00 mg. per kilogram of body weight.
[h] Endrin dosage of 800 mg. daily was administered intermittently on 11 days of the 22-day period and withheld on 11 days because of severe toxicity symptoms. Average endrin intakes for the entire 22-day period are 38.9 p.p.m. of the ration and 1.17 mg. per kilogram of body weight.

A comparison of the concentrations of endrin in the milk of cows fed endrin-sprayed hay (table 1) and that of cows fed endrin dissolved in soybean oil (table 2) indicates that endrin is excreted in the milk in higher concentrations when fed as a residue on sprayed hay than when fed as the technical material dissolved in soybean oil. Similar results have been reported for DDT (Ely *et al.* 1952). These results emphasize the hazards associated with the prediction of insecticide residues that may be encountered when information is not available for similar experimental conditions.

The average concentrations of endrin in the milk of the cows receiving the 600 and 800 mg. of endrin daily are not comparable with the other intakes in this study since these two dosages were not continuous during the feeding period. Both animals showed symptoms of toxicity, inappetence, and decreased milk production of such severity that dosages of endrin were discontinued for sufficient periods to permit remission of the symptoms. Both animals were subsequently placed on a lower dosage, as shown in table 2. Toxicity symptoms appear in the dairy cow when excretion of endrin in the milk is at a relatively low level. Kunze & Laug (1953) reported that small amounts of toxicant are stored in the body fat of rats fed endrin. Over 90% mortality of chicks fed 12 p.p.m. of endrin in the diet for 42 days was reported by Sherman & Rosenberg (1954).

The results of bioassays of milk samples are also shown in tables 1 and 2. Residues toxic to the pomace fly of the same general order of magnitude as the chemical analyses are indicated by these results.

SUMMARY.—Hay made during two seasons from alfalfa sprayed with 2.7, 6.6, and 7.8 oz. of endrin per acre, harvested 1 week after spraying and stored dry in bales for 6 months, had 2.8, 3.7, and 1.9 p.p.m. of endrin, respectively, when fed to dairy cattle.

Cows receiving the hay containing endrin residues of 1.9, 2.8, and 3.7 p.p.m. produced milk with <0.05, 0.14, and 0.15 p.p.m. of endrin, respectively. These results indicate that daily endrin intakes of greater than 20 mg. as a residue on sprayed forage are necessary for excretion of measurable amounts of endrin in the milk.

When feeding endrin dissolved in soybean oil to milking cows higher intakes were required to detect endrin in the milk than when feeding endrin residues on alfalfa hay. Toxic symptoms were noted in two cows receiving in excess of 1.5 mg. of endrin in soybean oil per kilogram of body weight.

Relatively low concentrations of endrin are excreted in the milk fat when toxicity symptoms appear in the animal.

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Susceptibility of European Chafer and Japanese Beetle Larvae to Different Strains of Milky Disease Organisms[1]

H. TASHIRO, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*[2]

Milky diseases of larvae of the Japanese beetle, *Popillia japonica* Newm., caused by *Bacillus popilliae* Dutky and *B. lentimorbus* Dutky are among the more successful biological-control agents against an insect. Larvae of the European chafer, *Amphimallon majalis* (Raz.), have been found susceptible to both these bacteria (Tashiro & White 1954). A comparison of infection rates in injection and feeding tests (Beard 1944, Tashiro & White 1954) indicated that the two species have similar susceptibilities to *B. popilliae*. Studies carried on in 1954 and 1955 by the writer indicated that the European chafer might be even more susceptible than the Japanese beetle to certain strains. In spite of the generally high susceptibility of European chafer larvae in the laboratory, the incidence of milky disease in these larvae in the field has been generally low, and areas of successful disease establishment have been limited.

Adams & Wheeler (1946) indicated that the establishment of milky disease in Japanese beetle larvae in the field is also slow in the more northern latitudes. Its low incidence in European chafer larvae may be explained also by the environment—that is, cooler soils in western New York during feeding periods of the larvae in fall and spring. It was considered desirable, therefore, to determine the relative susceptibilities of the two hosts to several strains of milky disease in parallel tests.

SOURCE OF LARVAE.—Only field-collected third-instar larvae were tested. Japanese beetle larvae were collected in the spring of 1954 from Windsor, N. Y., near areas where milky disease spore dust had been distributed in previous years. In these larvae the very low incidence of the disease with certain strains of organisms in the first tests suggested that the more susceptible individuals might have been eliminated by milky disease. Therefore, larvae for additional tests during the winter of 1954–55 and the spring of 1955 were collected at Waverly, N. Y., where there had been no known distribution of milky disease organisms. European chafer larvae were collected in the more recently infested areas surrounding Newark, N. Y., which were known to be free of milky disease.

BEHAVIOR OF LARVAE UNDER INCUBATION.—There was a decided difference in the development of larvae of the two species when incubated at relatively high temperatures. The Japanese beetle larvae were younger than the chafers at the start of the tests, but pupated sooner at certain periods. Neither species pupated after 3 weeks' incubation at 80° F. in January, but during February and March 50% of the Japanese beetles pupated within 3 weeks as compared with 2% of the chafers. During May, when the chafers were normally approaching pupation, their rates of development under incubation coincided. Within 3 weeks 72% of the Japanese beetle and 85% of the chafer larvae had pupated. It was felt that the Japanese beetle larvae, being more immature, would prove more susceptible than those of the chafer to certain strains of the organisms, but neither their age nor the rapidity with which they pupated appeared to influence their inherent susceptibility to the various strains of the milky disease.

SOURCE OF MILKY DISEASE ORGANISMS.—Three strains of the milky-disease organisms were tested against both insects in injection and feeding tests—two of *Bacillus popilliae*, referred to as the regular and DeBryne strains of type A, and one of *B. lentimorbus*, the *Amphimallon* type-B strain.

Spore dust prepared at the Moorestown, N. J. laboratory with diseased Japanese beetle larvae was used as the source of regular type-A strain for the feeding tests, and spores from Japanese beetle larvae that had become diseased through ingestion of the spore dust for the injection tests.

The DeBryne strain was originally obtained from the DeBryne-Wheeler plot (Tashiro & White 1954), where the incidence of type-A infections in European chafer larvae had been the most consistent. This appeared to be the type-A strain most virulent towards European chafer larvae. Spores from chafer larvae diseased after 7 to 15 passages through this host in other laboratory feeding experiments were used for both injection and feeding tests.

The *Amphimallon* type-B strain originally came from an abandoned hillside pasture in Port Gibson, N. Y. (Tashiro & White 1954). Spores obtained from European chafer larvae becoming diseased after 6 to 13 passages of this organism through this host in laboratory feeding experiments were used for both tests.

INJECTION TESTS.—Larvae of both species received approximately 100,000 spores per individual. Injections of 0.0035 ml. of spore suspension adjusted to contain 30 million spores per milliliter were made with a microinjector. Techniques used for preparation of suspensions and injections were as described by Dutky (1942). Injection was along the dorso-lateral region of the fourth and fifth abdominal segments.

For each test 100 larvae were injected with each strain. The larvae were then incubated in moist soil containing germinating domestic rye grass seed for food, in wooden trays with 50 compartments separated by metal cross-section dividers. One larva was placed in each compartment, which had a volume of 2 cubic inches. Two tests were conducted at 70° F., the first in the spring of 1954 and the second in the winter of 1954–55. Three tests were also conducted at 80°, the first two during the same periods and the third in the spring of 1955. Only two tests at 80° were conducted with the *Amphimallon* strain during the spring of 1955. Checks for each test included 50 larvae injected with sterile distilled water and an additional 50 noninjected larvae to check against injection techniques.

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[2] In cooperation with the Department of Entomology, New York State Agricultural Experiment Station, Geneva, N. Y.

Weekly or semiweekly examinations were made, and larvae showing gross symptoms of disease were removed from the trays. Periodic microscopic examinations of the blood of representative larvae were made to be sure that the disease was caused by the intended bacterial species.

As shown in table 1, the regular type-A strain produced nearly twice the incidence of disease in European chafers as it did in Japanese beetle larvae at both temperatures. The DeBryne strain showed even greater differences, being practically nonvirulent toward the beetle larvae. This difference was greater at 70° than at 80° F. The *Amphimallon* strain was more than twice as virulent to chafers as to the beetle larvae at 80°. Incubation beyond 3 weeks at either temperature showed no significant increase in the incidence of disease in either species. None of the check larvae were diseased at 70°. The limited amount of infection in the checks at 80° was probably caused by contamination in handling.

There was some indication that the Japanese beetle larvae from Windsor were less susceptible to the regular type-A strain than those from Waverly.

FEEDING TESTS.—Larvae of both species were incubated in moist soil containing approximately 1 billion spores per kilogram of air-dry soil. Type-A spore dust was mixed with the soil to obtain this concentration. European chafer larvae infected with the DeBryne strain in other feeding tests were held until maximum sporulation was obtained, and then thoroughly macerated, suspended in water to make spore counts, and mixed with the soil to obtain the desired concentration. Soil inoculations with the *Amphimallon* strain were made with chafer larvae that became diseased in feeding tests and handled in the same manner. As in injection tests, two trays of 50 larvae of each species were incubated in each test. Checks included larvae incubated in spore-free soil.

The DeBryne strain was highly virulent to chafers but practically nonvirulent to Japanese beetle larvae. Approximately the same differences in susceptibility were manifested by the *Amphimallon* strain at 80°. There was no disease in the checks in either species. Except in the regular type-A strain at 70°, there was very little increase in infection beyond the third week.

DISCUSSION.—The European chafer larvae were equally if not more susceptible than the Japanese beetle larvae to infection with the regular type-A strain at both temperatures and both methods of inoculation. The fact that this organism had been cultured in Japanese beetle larvae and not in the chafers indicates that it has a naturally high order of virulence towards the chafer.

The Japanese beetle larvae appeared practically immune to the DeBryne strain. The origin of this strain is somewhat conjectural, but it could have one of two sources. Probably it is the regular or *anxia* type-A strain, the organisms distributed in the field in 1945. If so, it is conceivable that as the organism passed through the chafer its virulence towards this host gradually increased but its virulence towards the Japanese beetle larvae diminished until it became almost nonvirulent. In feeding tests the *anxia* type-A strain obtained directly from Japanese beetle larvae has not been of any higher virulence than the regular type-A strain to European chafer larvae (Tashiro & White 1954). It is also possible that the type-A strain found at the DeBryne-Wheeler plot has no connection with the type-A strains used for inoculating the plot, since type-A infections have been found to predominate in a number of areas far removed from any known distribution of spores. Some type-A strain previously having as its host some native white grub may have been in this field and adapted itself to European chafer larvae.

The distribution of the *Amphimallon* type-B strain is fairly general in the older chafer-infested areas. There are also two possible explanations as to the origin of this strain. Tashiro & White (1954) treated several plots with type-B spore dust prepared from Japanese beetle larvae, but these plots have produced very few larvae with type-B infections. It is not likely that the organisms distrib-

Table 1.—Percentage of third-instar European chafer and Japanese beetle larvae becoming infected with different strains of milky disease organisms in injection and feeding tests at different temperatures.

Incubation Temperature (° F.)	Weeks After Treatment	Regular Type A		DeBryne Type A		Amphimallon Type B		Check	
		Chafer	Beetle	Chafer	Beetle	Chafer	Beetle	Chafer	Beetle
				Injection Tests[a]					
70	1	0	0	10	0	—	—	0	0
	2	2	0	59	1	—	—	0	0
	3	8	3	90	1	—	—	0	0
80	1	37	4	78	1	65	1	1	0
	2	58	38	99	5	87	34	4	0
	3	64	39	99	8	93	34	4	0
				Feeding Tests[b]					
70	1	0	0	2	0	—	—	0	0
	2	5	1	55	0	—	—	0	0
	3	12	10	81	1	—	—	0	0
	4	35	30	81	5	—	—	0	0
80	1	0	0	22	0	1	0	0	0
	2	50	36	95	1	81	3	0	0
	3	65	51	95	1	92	9	0	0
	4	79	55	95	3	—	—	0	0

[a] Injected at rate of 100,000 spores per larva.
[b] Larvae incubated in soil containing 1 billion spores per kilogram.

uted in these plots have become established and carried to the many areas where type-B infected larvae have been found. It is more probable that the *Amphimallon* strain, which shows greater virulence to the chafer than to the Japanese beetle, formerly had as its host some native white grub and has adapted itself to the chafer with increasing virulence through successive passages.

SUMMARY.—The susceptibility of third-instar larvae of the European chafer, *Amphimallon majalis* (Raz.), and the Japanese beetle, *Popillia japonica* Newm., to the regular and DeBryne type-A strains of the milky disease organisms *Bacillus popilliae* Dutky and the *Amphimallon* type-B strain of *B. lentimorbus* Dutky was compared in spore injection and ingestion tests at 70° and 80° F.

Infection by spore ingestion was slower than by injection but after 3 weeks the incidence of disease by the two methods of inoculation was about the same.

All three strains were more virulent to European chafer than to Japanese beetle larvae by both methods of inoculation. The DeBryne strain showed the greatest difference between the two hosts, European chafers being very susceptible but Japanese beetle larvae practically immune to it. The difference was greater at 70° than at 80° F. After 3 weeks incubation at 70°, 90% of the chafer larvae were milky through injection and 81% through ingestion of spores as compared with only 1% of the Japanese beetle larvae.

These tests indicate that all three strains give promise of being effective biological-control agents against the European chafer. It is probable that their lack of greater effectiveness in the field is due to low soil temperatures in western New York during the active feeding season in the fall and spring. From this standpoint the DeBryne strain shows promise of being the most effective.

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The Spotted Alfalfa Aphid and Its Parasites in the Mediterranean Region, Middle East, and East Africa[1]

ROBERT VAN DEN BOSCH,[2] *University of California Citrus Experiment Station, Riverside*

In February, 1954, a small yellowish-maculated aphid was observed to be causing damage to alfalfa (*Medicago sativa* L.) in eastern and central New Mexico, and in the following months it spread very rapidly over large areas in the southwestern United States. The aphid was first encountered in damaging abundance in California at Orita in the Imperial Valley on June 27, 1954, and soon thereafter became a widespread and serious alfalfa pest (Dickson *et al.* 1955). Initial examinations of aphid specimens by taxonomists showed this new alfalfa pest to be a species of *Therioaphis*,[3] seemingly the yellow clover aphid, *Therioaphis trifolii* (Monell), a species described in 1882 from material collected at Washington, D. C.

Despite the initial designation of the aphid as *Therioaphis trifolii*, the fact that it seemed to be spreading east and west from a focus in New Mexico, and its strong affinity for alfalfa as opposed to the clovers (*Trifolium* spp.) favored by *Therioaphis trifolii*, indicated that the new aphid might be (1) newly introduced and (2) at least a distinct biological form of *T. trifolii*. For these reasons, the taxonomists continued an intensive study of available aphid material to determine whether morphological differences existed between the alfalfa- and clover-feeding forms. This study eventually revealed very subtle differences between the two aphids, primarily in the number and arrangement of sensoria on the antennae. These same studies showed the new aphid to be identical with *Therioaphis maculata* (Buckton), an Old World species described in 1899 from specimens collected in Jodhpur, India (Dickson *et al.* 1955). In the spring of 1955 the common name "spotted alfalfa aphid" was proposed for *T. maculata*, the name "yellow clover aphid," by which it had previously been known, being already the common name used for *T. trifolii* (Dickson *et al.* 1955).

As the destructiveness of the spotted alfalfa aphid increased, interest arose as to the possibility of obtaining *Therioaphis* parasites in foreign areas, inasmuch as no parasite had been recorded from the new pest in the United States. Accordingly in the spring of 1955 a cooperative program was initiated between the U. S. Department of Agriculture and the University of California to undertake a search for natural enemies of *Therioaphis maculata* in Europe, the Middle East, Africa, and Asia, and to attempt the introduction and establishment of

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[3] According to Dickson *et al.* (1955) the following generic names have been applied to the "yellow clover aphids": *Myzocallis* Passerini, *Callipterus* Koch, *Therioaphis* Walker, *Triphyllaphis* Börner, *Pterocallidium* Börner and *Myzocallidium* Börner. In the current paper the writer follows Dickson in applying the generic name *Therioaphis* to the species under consideration

such natural enemies as might be found. Responsibility for foreign exploration was divided among Dr. Harry Parker of the U. S. Department of Agriculture Parasite Laboratory, Paris, France, who was to search western Europe; Dr. George Angalet of the U. S. Department of Agriculture, who was to search India and Pakistan; and the writer, who was to search the eastern Mediterranean, Middle East, and East African areas. As a result of this cooperative effort, three hymenopterous parasites, *Praon palitans* Muesebeck and *Trioxys utilis* Muesebeck (both Braconidae) (Muesebeck 1956) and *Aphelinus semiflavus* Howard (Eulophidae), were collected from *Therioaphis* spp. and are now in production in the insectaries of the University of California Biological Control Department at Riverside and Albany, California.

The exploration under discussion in this paper commenced in Italy in early June, 1955, and terminated in Eritrea (Ethiopia) about May 1, 1956. Countries visited during this period included Egypt, Cyprus, Israel, Yugoslavia, Turkey, Lebanon, Iran, Iraq, and Kenya, as well as Italy and Ethiopia.

Exploration was initiated in the eastern Mediterranean region because of the report in correspondence by Dr. I. Harpaz of the Hebrew University, Rehovot, Israel, that he had encountered parasites of *Therioaphis maculata* while studying the biology of the aphid in that country. Actually, the first parasite shipment was made from Italy, which was visited briefly by the writer on his way to the Middle East. The first large-scale search for parasites was initiated in Egypt, however, political considerations making it advisable to visit Egypt before going on to Israel.

The parasites discussed herein were identified by C. F. W. Muesebeck and B. D. Burks of the U. S. Department of Agriculture, Washington, D. C.; aphids were identified by R. C. Dickson of the Department of Entomology, University of California Citrus Experiment Station, Riverside.

A country-by-country discussion of collecting conditions follows, beginning with the activity in Egypt.

EGYPT.—This country was actually visited twice, first in June, 1955, and then during January and February, 1956, when both the spotted alfalfa aphid and the Egyptian alfalfa weevil, *Hypera brunneipennis* Boheman, were investigated.

Alfalfa is practically nonexistent in Egypt, the major forage plant being berseem (*Trifolium alexandrinum* L.), which is planted in the fall of the year and grown as an annual crop. At the time of the June, 1955, visit most of the berseem had been "dried up" or treated with cotton dust (DDT-BHC), as required by law to prevent build-up of the so-called cotton leaf worm, *Prodenia litura* Fabricius, and collecting conditions were very poor. *Therioaphis* was found in fair abundance at several places in the vicinity of Cairo, but no parasitized specimens were collected. Aphids collected from berseem in Egypt at this time have been identified as a mixture of *Therioaphis maculata* and *T. trifolii*. The return visit to Egypt in January, 1956, was even less fruitful than that of the previous June, for not a single aphid or parasite specimen was observed or collected, despite almost daily inspection of berseem fields and wild legumes such as bur clovers (*Medicago* spp.) and sweet clovers (*Melilotus* spp.).

As the later investigations in Israel showed parasites to be common in that country, it is difficult to believe that these species do not occur in neighboring Egypt. It is possible that the annual cultural pattern of berseem, which results in a clover-free period of about 4 months during the hottest part of the year, has a strongly disruptive effect on both aphid and parasite populations and that the parasites, which probably lag behind the aphid in their build-up, are more drastically affected by this factor and thus never attain high population levels.

CYPRUS.—On leaving Egypt in June, 1955, the writer proceeded to Israel via Cyprus. During a 2-day stopover on this Island, a number of alfalfa fields were visited in the vicinity of Nicosia, and although light aphid populations were encountered at several places, no parasites were observed. Again, as in the case of Egypt, it is the writer's opinion that all three parasite species probably exist on Cyprus, but for unexplained reasons are extremely rare. Specimens of *Therioaphis* collected from alfalfa in Cyprus were identified as *T. maculata*.

ISRAEL.—The search for parasites of spotted alfalfa aphid in Israel extended over a period of approximately 3 weeks, and in contrast to the unrewarding efforts in Egypt and Cyprus, results were most fruitful, all three parasite species being collected.

While in Israel the writer had an opportunity to visit much of the agriculturally developed area of that country, and spotted alfalfa aphid was encountered both in the hot, arid Jordan Valley and on the humid coastal plain. *Praon palitans*, the dominant parasite, was found in abundance in both areas. Most of the parasite material obtained for shipment was collected in the vicinity of Rehovot on the coastal plain, however. As indicated above, *Praon palitans* was the dominant species, 2,100 specimens being collected; *Aphelinus semiflavus* was second in importance, 260 specimens being obtained. *Trioxys utilis*, which later proved to be an abundant and at times dominant species in other countries, was rarely encountered in Israel, only eight specimens being obtained. The relatively important status of *Aphelinus* in Israel was also in contrast to anything encountered in later collecting.

According to Harpaz (1955), *Therioaphis maculata* is a minor and sporadic pest of alfalfa in Israel. This was the condition encountered by the writer in the summer of 1956, when only one of a large number of fields examined was found infested enough to be damaged. The aphid population in this sprinkler-irrigated field was very heavily attacked by a fungus disease which greatly reduced the severity of the infestation.

Aphid specimens collected from alfalfa and *Trifolium alexandrinum* in Israel have all been identified as *Therioaphis maculata*.

ITALY.—As indicated previously, the writer spent a brief time in Italy before going to Egypt. At this time, collecting was done in the vicinity of Sorrento in the company of S. E. Flanders of the Department of Biological Control, University of California. During this visit parasites of *Therioaphis* were readily collected, *Praon* sp. being obtained from *Therioaphis ononidis* (Kaltenbach) ex *Ononis* sp., and *Trioxys utilis* from *Therioaphis maculata*.

The second visit to Italy, which was prompted by the

earlier success at Sorrento, extended from about mid-July to mid-August, 1955. The writer had originally intended to return to the Sorrento area, but a survey in the vicinity of Rome immediately after arrival in Italy, in July, revealed much wider cultivation of alfalfa there and much more promising conditions for parasite collection than in Sorrento; consequently, it was decided to concentrate the collecting effort in the vicinity of Rome.

The search for parasites in Italy proved as fruitful as that in Israel, but revealed a much different quantitative relationship of the three species. This is reflected in the numbers of specimens shipped to Riverside, the totals being as follows: *Trioxys utilis*, 2,750; *Praon palitans*, 700; and *Aphelinus semiflavus*, 3.

In the period of approximately 1 month of collection and observation in Italy, not a single spotted alfalfa aphid infestation even approaching economic proportions was encountered. In one field, however, the cowpea aphid, *Aphis medicaginis* Koch (=*A. craccivora* Koch), was observed to have reached damaging abundance.

Aphid specimens collected from alfalfa at several localities in the Rome and Sorrento areas were identified as *Therioaphis maculata*, and, as mentioned above, *T. ononidis* was collected jointly with S. E. Flanders near Sorrento.

YUGOSLAVIA.—The writer visited Yugoslavia on his way from Italy to the Middle East, as it was felt that the area in the vicinity of Belgrade, having a climate distinct from that of the Mediterranean littoral, might offer unique collecting opportunities. The weather at Belgrade at the time of this visit was cool and humid, and the countryside was lush and green, strong indication of heavy rainfall in previous months. Spotted alfalfa aphid was extremely scarce, and intensive collecting during a week's time produced only 58 *Praon palitans*, about half of which were reared specimens. *Therioaphis* specimens collected from alfalfa in Yugoslavia were identified as *T. maculata*.

TURKEY.—Collecting in Turkey was carried out on the Anatolian Plateau in the vicinity of Ankara, where for the first time climatic conditions were encountered which approximated those of the arid southwestern United States. The winters at Ankara are relatively severe and the summers dry and mild, a climatic pattern which characterizes much of the plateau area of the Middle East. Alfalfa in the vicinity of Ankara is grown in small patches along the streams, heavier concentrations being located at Kirsehir and Konya to the east and south. In correspondence, Dr. Z. U. Düzgünes, entomologist at the University of Ankara, has reported *Therioaphis* as causing serious damage to alfalfa at Kirsehir in 1953.

Under conditions in the vicinity of Ankara in the late summer of 1955, spotted alfalfa aphid was found to be generally distributed and relatively abundant. Despite this aphid abundance, however, parasites were rare, and intensive collecting over a 3-weeks' period yielded only 41 *Praon palitans*, 3 *Trioxys utilis*, and 2 *Aphelinus semiflavus* from the field and from rearings.

Reasons for the scarcity of parasites in the Ankara area can at best only be speculated upon, the cold winters possibly having an adverse effect on them and the scattered and limited cultivation of alfalfa also possibly influencing their abundance.

Aphid specimens collected from alfalfa at Ankara were identified as *Therioaphis maculata*. *T. trifolii* and *T. luteola* (Börner) were also collected from *Trifolium* spp. in the vicinity of Ankara.

LEBANON.—This country was visited briefly in September, 1955, while the writer was in transit to Iran, and again in the spring of 1956, while in transit from Iraq to Egypt. No alfalfa was encountered on the western slope of the Lebanon, but there was considerable acreage in the Bekaa Valley to the east of the mountains. Although no infestations of economic importance were found in alfalfa fields in this area, one of the heaviest concentrations of parasites (all *Praon palitans*) encountered anywhere in the Middle East occurred in a field in the Bekaa. Because of the proximity of the Lebanese collecting area to Israel, however, only a single shipment of *Praon palitans*, totaling about 100 specimens, was sent to California.

During a brief stopover in Lebanon in March of 1956, a return visit was made to the Bekaa Valley, but apparently because of the earliness of the season spotted alfalfa aphid was extremely rare and neither aphids nor parasites could be collected at that time.

IRAN.—As Iran is a country to which alfalfa is indigenous and since it is a country of wide climatic and geographic variation, an effort was made to collect parasites and to observe spotted alfalfa aphid under as wide a variety of ecological conditions as possible; consequently, collections were made in literally all four corners of the country, as well as on the central plateau and on the tropical Caspian Sea Coast. The greatest amount of collecting was done in the vicinity of Tehran on the central plateau, and material collected in this region largely influenced the quantitative make-up of the parasite shipments sent from Iran, the totals being *Praon palitans*, 2,500; *Trioxys utilis*, 1,100; and *Aphelinus semiflavus*, 2. Most of the specimens were collected in the field, but a limited number of parasites, including the 2 *Aphelinus*, were reared.

As indicated in the totals above, *Praon palitans* was the dominant parasite on the central plateau. This condition, however, was not true in all areas, for this parasite was not collected at all on the hot, arid coastal plain near Ahwaz in southwestern Iran, *Trioxys utilis* being the only species encountered there. At the time of the Ahwaz collecting, spotted alfalfa aphid was quite rare and most of the *Trioxys utilis* specimens were obtained from a single small alfalfa patch in which a light aphid population was very heavily parasitized. The occurrence of *Trioxys utilis* in the Ahwaz area, which is climatically almost identical with the low desert areas of California, is an indication that this parasite is well adapted to this severely hot climate. On the other hand, the apparent absence of *Praon palitans* from this area is an indication that this parasite may never attain important status in the lowland desert areas of the southwestern United States.

Parasite collecting on the humid semitropical Caspian Sea Coast to the north of the Elburz Mountains more or less duplicated that of the Persian Gulf area, with only *Trioxys* sp. being encountered. As alfalfa and spotted alfalfa aphid were both rare in this region, however, all the *Trioxys* were obtained from a heavily parasitized population of *Therioaphis rhiemi* (Boerner) found on *Meli-*

lotus near the village of Shahi. It is not known whether the *Trioxys* attacking *T. rhiemi* is the same as *Trioxys utilis* as stocks of these parasites became mixed in the insectary at Riverside. The possibility that the two forms may at least be distinct biologically cannot be overlooked, however.

During the 2½ months of collection and observation in Iran, a large number of alfalfa fields were visited, and of these an estimated 10 to 20% were heavily infested with spotted alfalfa aphid. Except in one experimental field of an American variety of alfalfa and one very small patch of a native variety, these infestations did not appear to cause severe damage, thus suggesting that the native alfalfa varieties grown in Iran may have an inherent resistance to the aphid. In the case of the heavy infestation in the experimental planting mentioned above, the aphid population had passed its peak when the field was examined, but abundant honeydew and sooty mold gave ample evidence of the earlier damaging infestation. On the other hand, large numbers of coccinellids of several species and swarms of *Praon palitans* and *Trioxys utilis* adults in the field gave strong indication that these natural enemies, together, may have been responsible for the aphid "clean-up."

Aphid specimens collected from alfalfa in Iran were identified as *Therioaphis maculata*. In addition, as mentioned earlier, *T. rhiemi* was collected from *Melilotus* on the Caspian Sea Coast, while *T. trifolii* was collected from *Trifolium* sp. at Hyderabad, about 20 miles west of Tehran.

IRAQ.—Iraq was visited on two occasions, the first visit extending from late November to late December, 1955, and the second occurring during February and March, 1956. Search for parasites was concentrated in the vicinity of Baghdad, an area with a climate almost identical with that of the intensely hot and arid low desert of southern California. At the time of the late 1955 visit, the weather was cool and inclement, and spotted alfalfa aphid was rare in the field. As a result, parasite collection was very difficult and during the month's time only 30 specimens, all *Trioxys utilis*, were obtained, mainly through rearings.

During the second visit to Iraq much of the writer's effort was devoted to a study of parasites of the Egyptian alfalfa weevil, *Hypera brunneipennis* Boheman, and only a small number of *Trioxys utilis* were collected from spotted alfalfa aphid. As indicated above, populations of the spotted alfalfa aphid were generally low in the Baghdad area during the winter and early spring of 1955–56; however, in March, as temperatures began to rise, the aphid increased somewhat in abundance. Correlated with this increase was the appearance of the coccinellid *Coccinella 7-punctata* L., and an increasing incidence of fungus disease. These two biotic factors seemed to have a definite effect in slowing aphid build-up, but the writer's departure from Iraq in late March prevented any thorough study of this relationship.

As was mentioned above in the discussion of the role of *Trioxys utilis* in the hot coastal region of Iran, it seems significant that this parasite was again the only species to be found attacking spotted alfalfa aphid in an area of intense summer heat. This must be taken as strong evidence that this parasite is the only species adapted to a hot desert climate and may therefore be the only one which will become established in such areas in the United States.

Aphid specimens obtained from alfalfa in Iraq were identified as *Therioaphis maculata*.

KENYA.—Alfalfa is not widely grown in Kenya, and search for spotted alfalfa aphid and its parasites was limited to several fields in the Naivasha area and in the vicinity of Kitale on the Kenya-Uganda border. Spotted alfalfa aphid was not encountered in any of these fields, although both pea aphid, *Macrosiphum pisi* (Harris), and cowpea aphid, *Aphis medicaginis* Koch, were readily collected. This apparent absence of spotted alfalfa aphid from the field, and the lack of any knowledge of this insect among the Kenyan entomologists, seem to indicate that it does not occur in this country.

ETHIOPIA.—Ethiopia possibly represents the most southerly limit of spotted alfalfa aphid distribution in Africa, for the aphid was collected in the vicinity of both Addis Ababa and Asmara (Eritrea) during a brief visit in the country. Although the aphid was readily encountered in both places, it was not abundant at the time of writer's visit, and no parasites were collected. At Asmara, however, an Eritrean entomologist stated that in previous years he had observed parasites in considerable numbers during the summer months.

Aphid specimens collected from alfalfa in Ethiopia were identified as *Therioaphis maculata*.

DISCUSSION.—Rapid movement through 11 countries in as many months was not conducive to the recording of precise data on the spotted alfalfa aphid and its natural enemies; thus this paper is but a report on general observations. However, the months of collection and observation revealed certain aspects of aphid and parasite distribution, abundance, and behavior which bear further discussion.

In the area visited, the spotted alfalfa aphid was found as far west as Italy; as far north as a line running through Belgrade, Yugoslavia, Ankara, Turkey, and the Russian-Iranian border in the vicinity of Meshed, Iran; as far east as Meshed, which is near the Afghanistan border; and as far south as Addis Ababa, Ethiopia. Over this vast area, wherever sampling was done in alfalfa, this aphid was found, and during the time of the writer's activity in the field it was, in general, the predominant aphid. The two other common alfalfa-infesting species, *Aphis medicaginis* and *Macrosiphum pisi*, were found to be more abundant at certain places, but were not nearly so uniform in distribution. Despite its wide distribution and dominant status on alfalfa, *Therioaphis maculata* was encountered in damaging numbers in only three of the hundreds of alfalfa fields visited. In discussing this aphid with entomologists in the various countries, it was mentioned as an economic pest only in Israel and Turkey, and then as of minor importance. A lack of concern by the peasant farmers for insect damage could, of course, add to this impression of minor economic status of the spotted alfalfa aphid in a number of countries.

The reasons for the generally sub-economic status of spotted alfalfa aphid in the area under discussion could result from any one or a combination of the following factors: (1) natural resistance or tolerance of native alfalfa varieties to the aphid; (2) cultural practices

whereby frequent cutting of small, scattered alfalfa patches prevents build-up of high aphid populations, (3) a combination of biotic mortality factors, namely, parasites, predators, and fungus diseases, which were observed over much of the area.

The parasite complex attacking *Therioaphis maculata* was found to be composed of three species, *Praon palitans* Muesebeck and *Trioxys utilis* Muesebeck (both Braconidae), and *Aphelinus semiflavus* Howard (Eulophidae). These parasites varied in actual and relative abundance from area to area, being apparently of minor importance in some areas and of considerable importance in others.

The geographic variation in abundance and importance of the parasites could be an indication that the species are composed of "strains" or biological races which may vary in their effectiveness as parasites of *Therioaphis maculata*. This possibility is given some substance by certain biological and phenotypic evidence, i.e., the existence of a uniparental "strain" of *Aphelinus* in Israel and a biparental "strain" in France (collected by H. Parker), and the existence of dark and pale "strains" of *Trioxys* in Iran.

This strain picture could, of course, be complicated by parasites of species of *Therioaphis* other than *T. maculata* which are biologically distinct but morphologically indistinguishable from the latter. This possibility was mentioned earlier in the case of *Trioxys* sp. collected from *Therioaphis rhiemi*.

Finally, the indication that climate may have considerable influence on the parasites seems substantiated by the lone occurrence of *Trioxys utilis* in the hot lowlands of the Persian Gulf Coast and Tigris-Euphrates Valley and by the abundance of *Aphelinus* in the humid coastal region of Israel, in contrast to its rareness in all other areas.

SUMMARY.—The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), an Old World species, increased tremendously in abundance and spread rapidly over the southwestern United States following its discovery in New Mexico in 1954. Foreign exploration for parasites of the aphid was initiated as a joint effort by the U. S. Department of Agriculture and the University of California in the late spring of 1955. As a result, three hymenopterous parasites, *Praon palitans* Muesebeck, *Trioxys utilis* Muesebeck, and *Aphelinus semiflavus* Howard, were obtained and are now being propagated in insectaries in California. The current paper reports the distribution and abundance of *T. maculata* and the distribution and importance of the three parasite species in 11 countries in the eastern Mediterranean region, Middle East, and East Africa, based on observations made during an 11-months' assignment to these areas.

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Toxicity of Fifteen Organophosphorus Insecticides to Several Insect Species and to Rats[1]

H. R. KRUEGER and J. E. CASIDA,[2] *Department of Entomology, University of Wisconsin, Madison*

Organophosphorus insecticides may be very general or quite selective in their toxicity. Industrial screening for active materials usually involves many insect species since there is a need for both general and selective insecticides. Published data relating structure to toxicity with different insects is often difficult to interpret because of the great variety of bioassay methods and mortality endpoints. In only about two dozen published papers have the conditions of treating the insects been standardized to allow calculation of a median lethal dose expressed as μgm. of organophosphate per gm. of insect. Even where large series of organophosphates have been subjected to quantitative (μgm./gm.) study, the species used have been limited. Data in the available literature have been reviewed by Metcalf (1955) and Krueger (1956).

This investigation primarily concerns the determination of topical LD_{50} data (μgm./gm.) for 15 organophosphates of widely varying structure on several insect species and rats. The insects were reared under uniform laboratory conditions and were treated by the same individual with standardized techniques.

MATERIALS AND METHODS.—*Insects.*—German cockroaches, *Blattella germanica* (L.), were reared on dog biscuits (Ralston Purina) and 3-week-old males were used for testing. Pea aphids, *Macrosiphum pisi* (Harris), were raised on pea plants (Perfection variety) to adults in 7 days of 68° F. After treatment the adult female aphids were held at a temperature of 76° F. in vials containing a segment of pea plant. Black carpet beetles, *Attagenus piceus* (Oliv.) were cultured on a mixture of Gaines dog meal and 5% autoclaved Brewers yeast. Approximately 5 weeks at 76° F. were required for development of the fourth-instar larvae used for assay from the egg stage. The larvae of the southern armyworm, *Prodenia eridania* (Cram.), were reared at 76° F. on lettuce to the fourth-instar used for assay in 6 to 8 days. After insecticide application these larvae were held in 25×200 mm. test tubes without food, at which time mortality counts were taken. House flies (Chemical Specialties Manufacturers

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Table 1.—Comparative toxicity of *O,O*-diethyl *p*-nitrophenyl phosphate applied topically to several species of insects.

ORDER, FAMILY	SPECIES	DEVELOPMENTAL FORM	AVERAGE WEIGHT (MG.)	L.D.$_{50}$ (μGM./GM.)
Orthoptera				
Blattidae	*Blattella germanica* (L.)	adult	50	0.75
Gryllidae	*Nemobius fasciatus* (DeG.)	adult	50	0.5
Locustidae	*Melanoplus femur-rubrum* (DeG.)	adult	300	2.0
Hemiptera				
Miridae	*Neolygus quercalbae* Knight	adult	7	1.5
Lygaeidae	*Oncopeltus fasciatus* (Dall.)	adult	50	0.3
Coriscidae	*Coriscus eurinus* (Say)	adult	50	1.2
Phymatidae	*Phymata fasciata* (Gray)	adult	40	3.0
Homoptera				
Aphidae	*Macrosiphum pisi* (Harris)	adult	2.8	0.5
Coleoptera				
Chrysomelidae	*Crioceris asparagi* (L.)	adult	10	0.03
Chrysomelidae	*Crioceris asparagi* (L.)	larvae	20	1.5
Chrysomelidae	*Acalymma vittata* (F.)	adult	15	0.08
Chrysomelidae	*Diabrotica undecimpunctata howardi* Barber	adult	25	1.0
Cerambycidae	*Tetraopes tetrophthalmus* (Forst.)	adult	160	3.7
Meloidae	*Epicauta pennsylvanica* (DeG.)	adult	60	4.5
Cantharidae	*Chauliognathus pennsylvanicus* DeG.	adult	55	1.0
Dermestidae	*Attagenus piceus* (Oliv.)	larvae	10	3.0
Coccinellidae	*Hippodamia convergens* G.-M.	adult	10	0.75
Lepidoptera				
Arctiidae	*Euchaetias egle* Drury	larvae	150	1.5
Arctiidae	*Hyphantria cunea* Drury	larvae	450	0.5
Notodontidae	*Datana integerrima* G. and R.	larvae	30	0.75
Pieridae	*Pieris rapae* (L.)	larvae	175	0.75
Noctuidae	*Heliothis zea* (Boddie)	larvae	180	1.5
Noctuidae	*Prodenia eridania* (Cram.)	larvae	35	1.8
Lycaenidae	*Strymon melinus* var.-*pudica* (Hy. Edwards)	larvae	30	1.0
Pieridae	*Colias eurytheme* Bdv.	adult	70	1.6
Diptera				
Muscidae	*Musca domestica* L.	adult	20	0.5
Hymenoptera				
Apidae	*Bombus* spp.	adult	200	0.3
Formicidae	*Formica sanguinea* Latr.	adult	15	1.1

Association (C.S.M.A.) 1948 strain) were cultured using the official C.S.M.A. method. Four-day-old female flies were used for test and after treatment were given granulated sugar and held for 24 hours prior to recording mortalities. Eggs of the yellow-fever mosquito, *Aedes aegypti* (L.), were placed in distilled water containing finely ground Garver's dog biscuits and fourth-instar larvae were obtained in 5 to 6 days at 76° F. All toxicants for mosquito larvae were introduced into test solutions in acetone and the final acetone concentration adjusted to 0.72%. The fourth-instar larvae were transferred from the culture to the test solutions and held without food for 48 hours, at which time mortality counts were made. From six to nine replicates of 25 larvae each were tested for each dosage of insecticide. All compounds appeared to be completely soluble at the dosage effecting 50% mortality. Twenty-two species of insects were collected in insecticide-free field locations near Madison, Wisconsin in the summer of 1955. Following treatment with the insecticide in the laboratory these insects were held 24 hours without food prior to recording the mortalities.

Application methods.—Insecticide solutions in acetone were applied topically to weighed insects with a calibrated micrometer-operated 0.25 ml. tuberculin syringe fitted with a 27-gauge needle which was bent at a 90° angle. The volume of acetone applied varied from 0.8 to 2.6 mm.^{3} depending on the size of the individual insects. The German cockroaches and house flies were treated on the ventral abdomen whereas the other insect species were treated dorsally on the abdomen. Carbon dioxide was used where anesthetization was needed for accurate topical applications. At least six replicates of 10 insects each were used for each dosage. The average mortality from four to six doses, each varying by a factor of two, were plotted on log-probit paper and the LD_{50} values read directly from the curve. Mammalian LD_{50} values were determined by subcutaneous injection into adult male white rats (150 to 250 gms.). The LD_{50} values are only approximate since dosages were varied by a factor of 2.0 and the insecticides were injected in distilled water, propylene glycol or acetone depending on the solubility of the various insecticides.

RESULTS AND DISCUSSION.—Para-oxon was the most generally toxic of the compounds studied (tables 1 and 2). Median lethal dosages for insects varied from 0.3 to 4.5 μgm./gm. with the exception of the family Chrysomelidae. The adults of *Crioceris asparagi* (L.) and *Acalymma vittata* (F). were extremely sensitive with LD_{50} values of 0.03 and 0.08 μgm./gm. respectively, while other chrysomelids were less susceptible. Thus, although quite generally toxic to all the insects studied, para-oxon can vary fifty-fold in its effectiveness to insects from within the same family.

Rat subcutaneous toxicity figures (table 2) were in general agreement with those in the literature (Metcalf 1955). The insect species exhibited varying degrees of susceptibility to the organophosphates studied (table 2), the pea aphids being most susceptible followed by the

Table 2.—Comparative toxicity of 15 organophosphates to six insect species and to rats (LD_{50}).

Insecticide[a]	Topical LD_{50} as Micrograms per Gram					P.p.m. *Aedes aegypti*	μGm./Gm. (subcut.) White Rats
	Blattella germanica	*Macrosiphum pisi*	*Attagenus piceus*	*Prodenia eridania*	*Musca domestica*		
O, O-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate (Dipterex)	30	20	>1000	2	13	0.09	400
1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate (Phosdrin)	7	0.75	7.5	1.2	1.0	0.04	0.8
diethyl *p*-nitrophenyl phosphate (para-oxon)	0.75	0.5	3	1.8	0.5	0.01	0.5
diethyl 2,4,5-trichlorophenyl phosphate	60	2.5	160	6	4.5	0.045	150
O,O-diethyl *O*-5-(3-methylpyrazolyl) phosphate (Pyrazoxon)	70	30	>1000	>1000	150	6	7
O-ethyl *O*-*p*-nitrophenyl phenylphosphonate	2	1.2	10	1.5	1.5	0.03	0.6
diisopropylfluorophosphate (DFP)	160	5	>1000	90	12	0.9	1.4
O-ethyl *O,O*-bis (*p*-nitrophenyl) phosphorothioate	>1000	600	>1000	>1000	>1000	9	>100
O-2-(ethoxy)ethyl *O, O*-diethyl phosphate	45	20	120	140	30	0.17	500
O,O-diethyl *S*-(isopropylthio)methyl phosphorodithioate (Am. Cyanamid 12008)	15	0.75	20	85	2	0.001	2
S-(2-oxoureidoethyl) *O,O*-diethyl phosphorodithioate	180	0.5	960	>1000	120	4	9
O,O-dimethyl *O*-*p*-nitrophenyl phosphorothioate (methyl parathion)	2	0.5	1.5	3	1.5	0.0015	6
S-(1,2-bis(ethoxycarbonyl)ethyl) *O,O*-dimethyl phosphorodithioate (malathion)	120	0.75	10	18	30	0.04	1000
octamethyl pyrophosphoramide (schradan)	>1000	10	>1000	>1000	>1000	37	9
bis(isopropylamino)fluorophosphine oxide (mipafox)	20	20	630	80	7	9	75

[a] Dipterex, Am. Cyanamid 12008, malathion and schradan were 99+% pure: all others were technical purity.

house fly, German cockroach, armyworm larva and black carpet beetle larva. The order of general toxicity for the compounds studied based on the average LD_{50} for each compound against five species of insects (mosquito larvae omitted) was as follows: para-oxon > methyl parathion > *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonate > Phosdrin > malathion > diethyl 2,4,5-trichlorophenyl phosphate > *O*-2-(ethoxy) ethyl *O,O*-diethyl phosphate > Dipterex > DFP > Pyrazoxon > mipafox > *S*-(2-oxoureidoethyl) *O,O*-diethyl phosphorodithioate > schradan > *O*-ethyl *O, O*-bis(*p*-nitrophenyl)phosphorothioate.

No adequate explanation is available as to the mechanism of these species differences in susceptibility to organophosphorus insecticides. All the compounds studied appear to poison as anticholinesterase agents (Metcalf 1955, Casida 1956, Spencer & O'Brien 1957) but the insect cholinesterases vary greatly in substrate specificity and susceptibility to inhibitors (Casida 1955). Detoxication mechanisms may play a major role in these susceptibility differences (Casida 1956). The continuing development of insecticidal organophosphates increases the importance of investigations on the difference in mode of action of these varied toxicants on insects.

Summary.—The quantitative toxicity of 15 organophosphate insecticides to six insect species and to rats was determined. These toxicity data are reported as μgm./gm. for German cockroaches, pea aphids, black carpet beetle larvae, armyworm larvae, house flies and white rats and as p.p.m. in solution for mosquito larvae. Of these species, the pea aphids were the most generally susceptible and the black carpet beetle larvae the most generally resistant to the organophosphates. Malathion displayed the highest desirable toxicity ratio between insects and rats, while schradan was the most selectively toxic to rats. Because of its general toxicity, para-oxon was further studied with 21 other species of insects and the LD_{50} values were found to vary fifty-fold among these insects.

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SCIENTIFIC NOTES

Distribution of DDT-Dehydrochlorinase in Various Tissues of DDT-Resistant House Flies[1]

S. S. Miyake,[2] C. W. Kearns, and H. Lipke,
Department of Entomology, University of Illinois, Urbana

Sternburg and associates (1954) have shown that various strains of DDT-resistant (R) house flies (*Musca domestica* L.) contain a water-soluble enzyme, DDT-dehydrochlorinase, (DDT'ase) which catalyzes the dehydrochlorination of DDT to the non-toxic compound DDE. Since this enzyme could not be detected in DDT-susceptible (S) flies the Illinois workers hold that this detoxicating system plays a major role in the resistance phenomenon. Other workers, however, have interpreted various generalized physiological reactions as indicative of fundamental functional differences between the tissues of DDT-treated R and S flies, and have claimed that detoxication plays a relatively minor part in the survival of resistant strains (Pratt & Babers 1953, LeRoux & Morrison 1954, Smyth & Roys 1955, Weiant 1955, Weismann 1955). On the other hand, the demonstration that measurable amounts of DDT'ase were associated with given tissues obtained from R flies would strongly indicate that the possession of an active detoxicating system is sufficient explanation for any differences in gross physiological behavior between R tissues and those of S flies following treatment with DDT. In an attempt to resolve these conflicting suggestions and to gain further insight into the resistance phenomenon, experiments have been executed whose purpose was the localization of DDT'ase in the tissues of the house fly.

Materials and Methods.—The Synergist strain of DDT-resistant flies was used in this study.[3] The LD-50 for DDT applied to the mesonotum in acetone was in excess of 50 μg. per fly. The tissues assayed were cuticle, brain, muscle, fat body, ovaries, intestinal tract and haemolymph. The tissues were dissected in 0.9% NaCl held at 0 to 2° C. The dissected organs were blotted free of excess saline, pooled, and immediately frozen for future analysis. From 80 to 150 female flies furnished sufficient material for each experiment.

The source of cuticle was the dorsal area of the thorax and abdomen, and included the epidermis. The flight muscles of the thorax were used as a source of muscle. The Malpighian tubules were included in the intestinal tract preparations. Whereas the above structures of necessity contained small amounts of associated tracheal and nerve tissues, the brain, fat bodies, and ovaries were obtained largely free of other tissues. The haemolymph was withdrawn by slight suction applied to capillaries inserted into the area of the ptilinum. The contents of the capillaries were expelled and frozen immediately. No attempt was made to separate the plasma from the haemocytes. From 1 to 2 μl. could be collected from a single fly.

At the time of analysis, the samples were thawed, weighed, and homogenized in 1.2 ml. of cold distilled water. After centrifugation at 12,000×G the precipitate was discarded and the supernatant used for soluble protein and DDT'ase determination. Soluble protein was determined after dialysis against 0.02 M phosphate buffer by the biuret method of Robinson & Hogden (1940) using crystalline bovine serum albumen as the standard.[4] The volumes of all the reagents were reduced by one-half in order to conserve the fly extracts. DDT'ase was measured according to Sternburg *et al.*, (1954) either precisely as described or with all components and volumes reduced by one-half.

Results and Discussion.—The results presented in table 1 are the average of three independent experiments. When the specific activities are stated on the basis of μg. DDE fored per mg. soluble protein per 2 hours, it can be seen that the fat body and the nerve, as represented by the brain, contain the highest titers of enzyme. Cuticle, muscle, and haemolymph show intermediate levels while ovary and gut contain little or no DDT'ase. In the case of the intestinal tract, the low level of activity may merely reflect destruction of the DDT'ase system by the proteolytic enzymes also present in the gut extracts.

Table 1.—Average μg. of DDE formed by various tissues of DDT-resistant house flies in 2 hours at 37°C.

Tissue	Average Per Cent Soluble Protein	Specific Activity: Average μg. DDE per Mg. of Wet Weight	Specific Activity: Average μg. DDE per Mg. of Soluble Protein
Fat body	1.7	3.1	183
Brain	1.6	1.0	65
Cuticle	0.40	0.18	42
Haemolymph	1.5	0.40	26
Muscle	2.4	0.29	19
Ovary	8.5	0.18	2.1
Intestinal tract	2.0	0.1	1.0

The data show that with the possible exception of the intestinal tract and ovaries, DDT'ase is more or less ubiquitously distributed throughout the resistant organism. Sternburg & Kearns (1950) had first demonstrated DDE formation *in vitro* using homogenized cuticle preparations from resistant flies. Although the absolute amount of enzyme is low in this tissue, the small amount of soluble cuticular protein serves to explain the relatively high specific activity. The observation that the fat body mediates the greatest level of DDT degradation indicates a major role for this organ in detoxication mechanisms.

With respect to the high activity of the fat body, it is possible that lipoproteins assist in the solubilization of DDT, thereby increasing the rate of breakdown. Since lipoproteins are commonly associated with lipid-rich tissues, this may hold true for the nerve preparations as well. It has been shown by Lipke and coworkers (1956) that small quantities of lipoproteins, by virtue of their solubilizing properties, facilitate the action of DDT'ase to the extent where the enzymatic dehydrochlorination of DDT is rapid and complete. To speculate in physiological terms, these data suggest that undegraded DDT in R flies (Babers & Pratt 1953) is probably extracellular, or retained in a system where it is not capable of acting physiologically nor is it liable to degradation.

Pratt & Babers (1953) observed differences in the rate of leg tremors between R and S flies treated with DDT on the thoracic ganglion. Similarly LeRoux & Morrison (1954) have interpreted the differences in mortality observed between R and S flies treated with DDT internally in the head as indicative of the function of factors other than detoxication in the survival of R flies. Other workers have drawn similar conclusions based on measurements of the sensitivity of poisoned R and S flies to the taste of sugar (Smyth & Roys 1955, Weissmann 1955). In view of the demonstration of substantial levels of DDT'ase in the nervous system of R flies, and the absence of this system in S flies, none of the reports cited above in any way exclude the possibility of detoxication serving as the predominant factor responsible for these observed differences in nervous actions. Thus, in the case of the poisoned S flies, abnormal nervous patterns are manifest,

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[2] Present address: 3273 Lincoln Avenue, Honolulu 16, Hawaii. Part of a thesis submitted by Stanley S. Miyake in partial fulfillment of the requirements for the degree of Master of Science.

[3] Citrus Experiment Station, University of California, Riverside.

[4] The Armour Co., Chicago, Ill.

whereas the R flies by virtue of detoxication continue more or less normal nervous activity.

SUMMARY.—DDT-dehydrochlorinase activity has been determined in various excised tissues of the DDT-resistant house fly on a soluble protein basis. High levels were found in the brain and fat body, intermediate amounts in the cuticle, muscle and haemolymph, and little or none in the ovary and intestinal tract.

The amount of water soluble, biuret-positive protein has been determined for the above tissues and the significance of these findings with respect to the mechanism of resistance in the house fly is discussed.

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Effect of Chlordane on Broomsedge When Applied to Turf for Control of Japanese Beetle Grubs[1]

J. B. POLIVKA, *Ohio Agricultural Experiment Station, Wooster*

In 1950 an experiment was established in a golf course rough in which wettable chlordane powder mixed with milorganite was used at the rate of 0, 1, 5, 10, 15, 20, and 25 pounds of the actual toxicant per acre for the control of Japanese beetle grubs. Each treatment was applied to plots 10 by 10 feet and replicated four times. When these plots were surveyed for grub population each October, no difference in broomsedge stand, *Andropogon*[2] *virginicus* L., was noted until in the fall of 1955. At that time it was possible to pick out the untreated plots and the various levels of chlordane plots by the variability in the broomsedge stand. All plots contained blue grass but the 25-pound rate chlordane plots were the only ones that did not contain any broomsedge. These high chlordane plots appeared greener than the other plots probably because of the absence of the broomsedge.

The stand of broomsedge was estimated in the different chlordane plots on the basis of the stand in the untreated plots which was considered 100%. The average comparative differences are shown in table 1.

Table 1.—Average comparative stand of broomsedge in the different chlordane plots in October 1955.

POUNDS OF ACTUAL CHLORDANE PER ACRE	ESTIMATED STAND OF BROOMSEDGE AS COMPARED WITH UNTREATED (PER CENT)
25	None
20	5
15	20
10	40
5	60
1	75
0	100

When these plots were sampled again in October of 1956, the differences in the broomsedge stand in the different treatments were similar to those noted in 1955. Since all of the plots received equal amounts of milorganite and since the only variable factor was chlordane, it must be assumed that the chlordane was responsible for the difference in the stand of broomsedge.

[1] Accepted for publication February 5, 1957.

[2] The genus *Andropogon* is commonly known as beard grass.

Occurrence of Corn Borer in Potato Tubers[1]

MAURIE SEMEL, *L. I. Vegetable Research Farm, Riverhead, New York*

The potato is considered to be one of the principal host plants of the European corn borer, *Pyrausta nubilalis* (Hbn.), on Long Island (Dohanian 1934) and elsewhere in the United States (Clark 1939, Caffrey & Worthley 1927, Jones *et al.* 1939). A thorough examination of past records and reports, however, has failed to disclose any information that the borer was ever found to attack the potato tuber. This note records such a finding on Long Island during 1956.

Although the larva of the corn borer generally confines its feeding to the above-ground portions of the host plant, underground parts may be attacked as well. Hodgson (1928) noted such injury to the fleshy, enlarged taproot of the beet and mangel. Most of the injury was limited to the crown area of these crops, but, occasionally, the larvae tunnelled throughout the root.

The presence of borer larvae in potato tubers is, apparently, an unknown, or, at most, an extremely rare occurrence. Percentage-wise, the number of tubers found to be infested was extremely small, but the important fact is that infested tubers could be found upon very careful examination. Apparently three factors, operating in consort, were responsible for this unusual occurrence. These may be summarized as follows: An extremely heavy population of corn borer; the rather extensive use by growers of vine-killing chemicals in late August and early September to prevent late blight infection of the tubers; and the occurrence of several heavy, washing rains preceding harvest which made the hilling of potato rows difficult or impossible, and led to the exposure of a large number of tubers.

Potato vines were moderately infested by the first-generation borers but the infestation was not destructive. A large second brood was generated, however, and a rather severe infestation resulted. A survey of 10 fields in the Riverhead, N. Y. area revealed that all fields were infested to a greater or lesser degree. An average of 43.6% of the plants in these fields was found to be

[1] Accepted for publication February 6, 1957

infested with an average of 336 larvae per 100 plants. In some instances, larvae were found in the stems 3½ inches below the soil surface. Of the hills examined, none were found to contain infested tubers at this depth. As a matter of record, only one tuber was found to be larva-infested. In this single instance, the tuber was so situated that a portion of its surface was protruding through the soil. The exposed portion was green in color and such a tuber would normally be considered a cull.

In summary, it appears that the infestation of potato tubers by the corn borer was a chance and rare occurrence in which the larvae, abundant in number, and seeking more favorable environmental conditions due to the destruction of the potato vines by top-killing chemical compounds, found more succulent foodstuff in the form of exposed tubers.

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Demeton Residues in Peaches[1]

JACK E. FAHEY and D. W. HAMILTON,
Entomology Research Branch, Agr. Res. Serv., U.S.D.A.

Although many of the phosphate insecticides have been recommended for control of insects on peaches, no recommendation or tolerances have been established for the use of demeton on peaches. This insecticide is being generally recommended for control of mites and aphids on apples and pears and possibly could be used for control of these pests on peaches. With this probability in mind an experiment was undertaken to determine the magnitude of demeton residues in peaches and to study the rate of degradation of these residues.

A plot of four Elberta peach trees was treated with 0.75 pint of demeton (26.2% active ingredient) to 100 gallons of spray on June 14, July 5 and July 26. Samples for residue analyses were collected following the July 26 spray and on August 1, 8 and 15. The demeton residue was determined by the anticholinesterase method.

Table 1.—Demeton residues in peaches, 1956.

DATE SAMPLED	P.P.M. OF DEMATON	
	Whole Peach	Pits Excluded
July 26	2.94	3.68
August 1	.95	1.13
August 8	.75	.84
August 15	.18	.21

Table 1 shows the residue of demeton found in the whole peach and with pits excluded. The final residue, 0.18 p.p.m. of demeton 21 days after spraying, is less than the tolerance (0.75 p.p.m.) of demeton residues permitted in apples and pears.

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An Undescribed Species of *Lorryia* (Acarina, Tydeidae) Causing Injury to Citrus Trees in Morocco[1]

W. A. SMIRNOFF, *Entomologist, Vegetation Defense Service, Ministry of Agriculture and Forestry, Morocco*

In autumn of 1955, we observed in certain orchards of the Rharb and Casablanca regions the appearance of numerous colonies of mites upon Citrus trees infested with the coccid *Saissetia oleae* Bern.

Since the mites were found in large numbers around the shields of *Saissetia oleae* females (fig. 1) we were inclined at first to consider them as predators of this scale. We have established the fact that these concentrations result from the attractiveness of the sugar excretions of the scale. Usually the large amounts of honeydew discharged by *Saissetia oleae* cause the formation of an abundant fumagina, a black film covering the foliage and twigs of the infected trees. In the present case the fungus most frequently found is *Fumago vagans* Auct.[2,3]

We also observed that the mites were equally attracted by the spermogonies (elastic filaments stretching forth from the mycelian carpet of the fungus) on the extremities of which sugary droplets of honeydew concentrate.

Mr. Cooreman, to whom we sent the specimens for determination, kindly informed us that it belonged to the family Tydeidae, was an unknown species of the genus *Lorryia*. We intend to describe it in a forthcoming publication. (A species of *Lorryia* with clublike posterior setae reported to be injurious to citrus in Valencia, Spain, has been sent through the agency of the Department of Biological Control, University of California, to E. W. Baker for determination.)

Mr. Cooreman also informed us that bibliographical data concerning *Tydeidae*, found on citrus trees, are very limited. In California *Tydeus californicus* (Banks) has been observed to feed on citrus leaves (Fleschner & Arakawa 1953).

Massive infestation by this mite, which may prove to be economically harmful, having been observed in certain zones of Morocco, it becomes necessary to give preliminary information concerning *Lorryia* sp. so that swift measures could be taken against it.

We shall simply state that the mite is of a pale lemon yellow color, darkening in a very slight, hardly visible way on the dorsal part. The eggs are oval, white, faintly translucent, yellowing very slightly when the eggs are ready to hatch. The female lays her eggs rather close one to the other, vertical wise, the narrow end directed downwards. The eggs are often laid in two or three layers. The incubation period is of 3 to 4 days. Larvae and nymphs are of a whitish color, becoming yellower while the nymph is molting into an imago. A generation lasts 12 to 14 days. We have been able to observe the beginning of the *Lorryia* infestation since autumn 1955. Colonies occurred round the females of *Saissetia oleae*. A few weeks later their numbers were such that all the foliage and branches of the host-plant were infested.

The swarming mites were particularly visible at the base of the twigs, the petioles of flowers and the various asperities of the branches. On the green background of the bark, yellow stains of 2–3 cm. in diameter, formed by adult *Lorryia* and the white stains made by larvae and their exuviae showed very clearly.

Premature sclerification of the green branches, followed by desquamation was observed on the spots where the *Lorryia* concentrated.

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[2] We are indebted to Mr. Rieuf, pathologist of the Vegetation Defense Service in Rabat, for the photographs of the scale insects and for the determination of the fungus.

[3] *Fumago vagans* Auct. mycelium with dematiaceous hypha, few and sparse conidia of the *Brachysporium* Sac. type with spermagonies looking like thick hair enlarged in the middle, neck-shaped in their upper part, the edges of the opening being fimbriated, unicellular spores, rounded on both edges, measuring 2-4×1.5-2. Fructification of the *Antennaria* Ik ex type.

FIG. 1.—Concentrations of *Lorryia* sp. adjacent to an adult *Saissetia oleae* Bern. on citrus.

The following winter they appeared in small spots at the top of the fruit-stems and the forming fruits. In spring 1956, the infestation became particularly dense. It will be interesting to note that this increase coincided with the appearance of larval scale in their third stage and of young females discharging abundant honeydew. Noticeable colonies of *Lorryia* fixed themselves during this period on the lower part of citrus leaves and started laying.

The colonies generally settle on the lower part of the leaf, forming plate 2–3 cm. long and 1 cm. wide near the central rib of the leaf. The larvae, on hatching, remain there until their first molt, then abandon the leaves.

No traces of sucking or any other pathological phenomena was observed on the infested leaves. In the end of May, we observed that *Lorryia* sp. were actively migrating towards the neighboring citrus trees, even those free of *Saissetia oleae*.

At the end of July, *Lorryia* sp. appeared upon young fruit and in most cases the females began to lay eggs. A large number in all stages of growth sheltered under the cover of sepals, fruit and under their peduncles and began to prick and suck the tissues of the young fruit. This injury results in a ring of dead brown tissue, enlarging as the fruit grows. Damage thus caused is similar to that occurring through thrips under the same conditions.

It is evident that this *Lorryia* sp., sporadically and in circumstances still to be determined, lives phytophagously. Infestation of the orchard progresses swiftly. Three to five months are sometimes sufficient to *Lorryia* to infest a citrus orchard of around 100 hectares.

We have observed that they first appeared in areas treated with the ester of thiophosphorate. It will be equally interesting to note that we found no specific predators of this *Lorryia* and that it is not attacked by the usual predators of citrus mites such as *Scymnus pallidivestis* Muls., *Scymnus subvillosus* Goeze, *Triphleps minuta L.*, and cecidomyid larvae.

CONCLUSION.—*Lorryia* sp. is an undescribed species of Acarina up to the present unknown in Morocco. It feeds usually on the honeydew given off by *Saissetia oleae;* during development this species may become phytophagous. After experimentation with different acaricides and ovicides for control, we advise dusting with a mixture of sulfur and inactive materials (lime, dust, talc . . .), during early spring.

When the fruit is ripening and there exists danger of leaf burning as a result of temperature, sulphurous applications can be made by spraying with Aramite (sulfite of chloretyl and butyl phenoxy isopropyl-6^{0}cm3/100 l. of water).

In order, at the same time, to control *Saissetia oleae* and other Diaspidinae we recommend the incorporation in the mix of 1.5 to 2.5 per cent white oil, which permits a better spreading of the acaricide.

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Malathion Residues on Vegetable, Berry, and Tobacco Crops[1]

R. L. WALLIS, FLOYD F. SMITH, HELEN G. WHEELER, and EDGAR A. TAYLOR[2]

Entomology Research Branch, Agr. Res. Serv., U.S.D.A.

Experiments were conducted at the Agricultural Research Center and the Maryland Agricultural Experiment Station, Beltsville, Md., in 1955 and 1956 to determine the amount of malathion deposited on vegetable, berry, and tobacco crops under field conditions and its subsequent deterioration. Residues on tobacco were studied as a possible hazard to workmen handling the crop. Previous experiments on residues of malathion resulting from applications on several vegetables were reported by Smith *et al.* (1954, 1955).

Malathion was applied in the field at the rate of 1¾ pounds per acre in dusts, and in wettable-powder and emulsion sprays. Rotary dusters and hand sprayers were used, the sprayers with a pressure of approximately 60 pounds per square inch. Measured quantities of the insecticides were applied to a given length of row.

Samples of edible portions of leafy and fleshy vegetables and samples of fully developed tobacco leaves were harvested as soon as the sprays dried or within 1 hour after treatment and immediately extracted with redistilled carbon tetrachloride. The extracts were then analyzed, always on the day the samples were collected. The analyses were made by the colorimetric method developed by the American Cyanamid Company (Norris *et al.* 1954), and the results were corrected on the basis of samples of untreated materials. This method is applicable to the analysis of a wide variety of plant materials. When the residue was heavy, additional samples were harvested at intervals of 1 or more days and analyzed to determine the decline.

Results given in table 1 show that residues of malathion were higher and remained longer on leafy vegetables and tobacco than on berries and fleshy vegetables. In general, the residues were about the same with the different formulations, but those from emulsions were reduced the fastest. In most tests the residues on leafy vegetables remained above 8 p.p.m. for 3 days or longer when no rainfall occurred, and for as long as 14 days on parsley.

Rainfall was a factor in reducing residues. After 14 days residues from plots of parsley that were protected from rainfall were 11 to 15 p.p.m. but from parsley plots exposed to 0.74 inch of rain they were 2 to 11 p.p.m. Washing samples of parsley for 1 minute in tap water 1 hour after treatment reduced the residue from 36 to 3 p.p.m. or lower.

In general the residues on fleshy vegetables were very low 1 hour after treatment. Only on okra was there more than 8 p.p.m., and washing in tap water gave a reduction from an average of 15 p.p.m. for the three formulations to 0.4 p.p.m.

The residues on berries were moderately high 1 hour after treatment in all tests, but dropped to below 8 p.p.m. within 1 day.

Malathion residues on tobacco (variety Virginia Gold, a cigarette tobacco) were studied to determine the hazard to workers handling the crop. Large amounts were deposited, and they were reduced at a slower rate than on leafy vegetables and berries. The residue remained above 8 p.p.m. for more than 5 days, but after 8 days was reduced by rainfall. Succeeding treatments as the season progressed left higher residues. Those from treatments

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[2] The writers appreciate the cooperation of I. C. Haut, L. P. Ditman, and S. H. Todd of the Maryland Agricultural Experiment Station for making available several crops used in these residue studies.

Table 1.—Residues of malathion on vegetable, berry, and tobacco crops at intervals after application in different formulations in 1955 and 1956.

Crop	Date of Treatment	Interval Between Treatment and Harvest	Rainfall (Inches)	Residue (p.p.m.) Dust	Wettable Powder Spray	Emulsion Spray
Leafy vegetables:						
Chicory	June 12	1 hour	0	36.1	27.5	37.4
		1 day	0	12.3	12.9	11.0
		3 days	0	2.3	6.7	3.8
Collards	June 12	1 hour	0	11.6	17.4	3.0
		1 day	0	12.9	13.9	4.0
		3 days	0	3.0	4.0	1.0
Endive	June 12	1 hour	0	12.9	20.8	19.5
		1 days	0	19.2	18.6	23.5
		3 days	0	13.4	8.1	9.6
		8 days	1.92	0.4	8.0	0.3
Land cress	June 12	1 hour	0	24.9	21.1	23.8
		1 day	0	17.5	26.2	20.2
		3 days	0	18.1	22.7	9.8
		8 days	1.92	0	1.5	0
Leaf lettuce	June 19[a]	1 hour	0	16.3	18.1	26.1
		1 day	0	16.6	19.9	28.9
		3 days	0	13.5	15.8	16.9
		7 days	0	3.3	8.5	13.2
	June 19	7 days	0.58	0.9	0.5	4.5
Parsley	June 25	1 hour	0	27.8	40.4	36.2
		1 day	0	30.2	33.2	24.7
		3 days	0.74	17.3	33.4	29.2
		7 days	0.74	13.1	24.7	23.3
		14 days	0.74	2.4	11.4	9.2
	Aug. 1[a]	1 hour	0	55.7	62.2	35.7
		7 days	0	17.7	27.3	14.9
		14 days	0	11.0	15.2	15.3
		1 hour[b]	0	1.2	2.2	3.0
Swiss chard	June 25	1 hour	0	15.6	38.4	29.5
		1 day	0	27.2	54.0	25.1
		3 days	0.74	0.4	0.4	0.2
	July 30	1 hour	0	17.8	16.1	28.6
		1 day	0	14.3	14.8	15.0
		3 days	0.01	8.0	9.8	5.0
		8 days	0.12	0	0	0
Turnip greens	Oct. 9	1 hour	0	13.5	13.6	11.2
		1 day	0	17.4	16.4	13.7
		3 days	0	18.3	19.9	11.5
		7 days	0	9.2	12.1	4.3
		14 days	.24	0	0	0
Fleshy vegetables:						
Carrot	July 31	1 hour	0	.5	1.0	.1
Cucumber	Aug. 23	1 hour	0	2.9	.8	1.3
		1 day	0	.5	.2	T
		4 days	.21	.1	T	T
Kohlrabi	June 12	1 hour	0	1.7	1.6	1.6
		1 day	0	1.6	1.0	.9
		3 days	0	.4	.2	.2
Okra	Aug. 25[c]	1 hour	0	3.9	19.8	15.2
	Aug. 30[c]	1 hour	0	7.3	18.8	17.6
	Aug. 23	1 hour[b]	0	.8	.5	T
		1 hour	0	5.0	6.2	4.3
		1 day	0	3.0	4.9	1.1
		4 days	.21	0	.3	0
Parsnip	Oct. 2	1 hour	0	.6	.8	.4
Radish	June 19	1 hour	0	1.7	2.7	5.1
		1 day	0	.6	1.7	2.0
Rhubarb	July 12	1 hour	0	2.3	5.9	6.7
		1 day	0	1.4	3.5	3.6
Summer squash	July 25[c]	1 hour	0	2.2	2.5	—
		1 day	0	0	.6	—
		1 hour[b]	0	0	0	—
	July 16	1 hour	0	3.4	4.0	3.7
	July 18	1 hour	0	2.1	2.8	3.0
Berries:						
Blackberry	July 10	1 hour	0	5.5	10.0	10.6
		1 day	0	2.5	—	3.2
	July 12	1 hour	0	7.5	10.1	9.3
		1 day	0	7.3	5.2	4.5
	June 17	1 hour	0	11.1	9.1	15.7
		1 day	.17	3.5	5.2	4.8
Black raspberry	June 26	1 hour	0	9.1	9.9	9.8
		1 day	0	3.9	2.6	.7
	June 28	1 hour	0	9.0	9.6	11.0
		1 day	0	5.1	5.3	2.9
Red raspberry	June 26	1 hour	0	5.6	6.4	4.0
		1 day	0	5.1	5.3	.6
	June 28	1 hour	0	11.6	10.3	9.8
		1 day	0	4.8	6.1	4.4
Tobacco	Aug. 7	1 hour	0	12.7	13.0	16.9
	Aug. 13	1 hour	0	18.3	16.3	17.0
		1 day	0	13.2	13.3	13.4
		3 days	.03	.9	7.8	5.4
	Aug. 24	1 hour	0	29.8	27.4	32.4
		3 days	.21	17.5	20.0	14.0
		5 days	.21	9.9	20.3	5.1
	Sept. 10	1 hour	0	32.8	52.8	36.9
		4 days	0	49.2	36.6	24.7
		8 days	.63	2.3	5.2	2.5

[a] Plots protected from rain.
[b] Samples washed for 1 minute.
[c] Indicates treatments were applied in 1955.

made on August 7 ranged from 13 to 17 p.p.m., and those from similar treatments made on September 10 left from 33 to 53 p.p.m. It is believed that this increase is due to the decrease in the weight of the tobacco leaves with aging. No malathion residues have been reported on the cured tobacco and it is unlikely that this insecticide would persist through the curing, redrying, and aging processes.

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Peanuts as Substitute Food for the Large Milkweed Bug[1]

Hubert Frings, Mable Frings, and Franklin Little,
Pennsylvania State University, University Park

Rearing of the large milkweed bug, *Oncopeltus fasciatus* (Dall.), on blanched peanuts instead of milkweed seeds was reported from this laboratory previously (Frings & Peissner 1952). Continued use of peanuts for rearing milkweed bugs in our laboratory indicated that they were not always satisfactory. As a consequence, some investigations were made on the value of peanuts as food for these bugs and the results are reported here.

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The first experiments were designed to get comparative data on growth of the insects on blanched peanuts and milkweed seeds. The bugs were reared in quart jars—50 eggs in each jar at the start—where they were given water and an abundance of either peanuts or milkweed seeds. The temperatures in the laboratory were 25° to 28° C. and R.H. 35% to 45% throughout. The results of these tests are presented in table 1.

The results with milkweed seeds are in good agreement with those of Andre (1934). The bugs grew more slowly and with greater variability in rate on blanched peanuts than on milkweed seeds, and survival was poorer. Egg production by bugs fed on peanuts was about $\frac{1}{3}$ to $\frac{1}{4}$ that of bugs fed on milkweed seeds. These results show that blanched peanuts may be substituted for milkweed seeds, but that one must start with a group of eggs equal to approximately twice the number of adults desired. Apparently blanched peanuts are near the lower limit for growth for some nutritional factor or factors. This belief is supported by the observation that even small quantities of milkweed seeds added to peanuts will allow satisfactory growth and survival.

Table 1.—Days needed to attain successive moults in *Oncopeltus fasciatus* when fed abundant milkweed seeds or peanuts.

Moult	Days Needed on	
	Milkweed Seeds	Peanuts
1st	6–7	6–7
2nd	10–12	10–14
3rd	14–16	17–22
4th	19–22	22–33
5th (adults)	27–34	33–51
No. of eggs at start	700	1,200
Per cent surviving to adulthood	65%–90% (mostly 70%–80%)	30%–75% (mostly 45%–60%)

During these tests, it was found that some batches of blanched peanuts failed to support the bugs at all. Blanched peanuts are heat-treated to remove the seed coats. This suggested that the treatment of the peanuts destroyed some nutritional factor. Accordingly, raw peanuts were substituted for blanched peanuts. These gave much more uniform results, but growth rate and survival were still within the limits previously found.

A preliminary study was made on the quantities of peanuts needed for "normal" growth. For this, bugs from 50 eggs were given 1, 5, 10 or 20 peanuts respectively, renewed daily, while similar controls were given an equivalent volume of milkweed seeds. There were three jars of 50 eggs for each level. The rates of growth on both were "normal" for the type of food, but survival was only 27% with one peanut and 50% with the equivalent in milkweed seeds. With five or more peanuts or milkweed seeds equivalent to this, survival was also "normal."

A preliminary study was also made on the effects of renewing the food at various intervals of time, with an abundance always available. Twenty raw peanuts were used per jar of 50 eggs, and these were renewed: daily; every third day; every week; once a month; or not at all. The controls were given an equivalent volume of milkweed seeds, renewed similarly. Growth was "normal" for the type of food in all cases, but survival increased from 30% to 70% on peanuts and from 65% to 95% on milkweed seeds with decreasing changes of food. The highest survival was found when the food was not changed at all. This would indicate that a cafeteria style feeder, as described by Worthley (1943), is probably best for these bugs.

With the thought in mind that adaptation to feeding on raw peanuts could occur, eggs from bugs reared on peanuts were used for offspring to be reared similarly. The only change observed, in five generations on peanuts, was that these bugs grew more slowly when placed on milkweed seeds, than those from parents reared on milkweed seeds. There was no apparent improvement in growth rate or survival in bugs reared on raw peanuts with continued "selection" for five generations. At the end of the fifth generation, unfortunately, the culture was lost through an accident not related to the experiments.

Raw peanuts can, therefore, be regarded as a substitute food for the large milkweed bug, but not as satisfactory as milkweed seeds. The most practical uses for raw peanuts would probably be to support a colony of bugs if milkweed seeds became exhausted or to extend a dwindling supply of milkweed seeds. Raw peanuts are much cheaper than milkweed seeds, if one has to pay workers to gather the seeds, and might thus be used to reduce the cost of rearing. There apparently are differences in blanching methods which make it advisable to use raw rather than blanched peanuts.

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Experiments with Kelthane for the Control of Citrus Red Mites[1]

Herbert Spencer and Allen G. Selhime,
Entomology Research Branch, Agr. Res. Serv., U.S.D.A.

Control of the citrus red mite, *Metatetranychus citri* (McG.), in the Southeastern States has been increasingly difficult in the last 15 years. Before that time oil emulsions were in general use for control of scale insects, and the applications against scales were also effective against these mites. When grove owners began to use copper, zinc, and manganese compounds in sprays for diseases or correction of minor-element deficiencies, heavy infestations of citrus red mites occurred (Spencer & Osburn 1949), and when parathion was substituted for oil emulsion as a scalicide red mite infestations became even heavier (Spencer & Norman 1952).

This aggravated problem could not be solved by adding oil emulsion to the summer sprays of wettable sulfur plus parathion, because sulfur and oil are incompatible. Moreover, extra oil emulsion sprays in the program were expensive. Acaricides that were effective, inexpensive, safe, and compatible with the sulfur-plus-parathion combination were therefore sought.

In 1955 emulsion and wettable-powder sprays of Kelthane were compared with Aramite and oil sprays, the acaricides recommended by the Florida Citrus Commission (1955), in tests conducted on Temple oranges near Fort Pierce. Each treatment was applied to single-tree plots replicated 10 times, as described by Spencer & Osburn (1948). The 90% cream-type oil emulsion was one widely used in Florida. The oil had a viscosity of 73 to 78 seconds Saybolt and unsulfonated residue of 90 to 92%.

The acaricides were added to the post-bloom combination spray, which contained in each 100 gallons 1 pound of 15% parathion wettable powder as a scalicide, $1\frac{1}{2}$ pounds of 53% basic copper sulfate as a fungicide, 1 pound each of 52% zinc oxide and manganese oxide for prevention of minor-element deficiencies, and 5 pounds of 90% wettable sulfur for control of rust mites. These materials were used on all trees, except as follows: The sulfur was omitted in the oil emulsion spray, $2\frac{1}{2}$ pounds of man-

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Table 1.—Numbers of citrus red mites on 250 leaves of Temple orange trees that were treated with post-bloom combination sprays containing Kelthane or other acaricides, 1955.

Acaricide in 100 Gallons of Combination Spray	Active Stages						Eggs					
	April 6[a]	May 19	June 8	July 25	Sept. 13	Nov. 18	April 6[a]	May 19	June 8	July 25	Sept. 13	Nov. 18
None	10	3	3	197	216	105	69	6	30	387	232	196
Kelthane EC, 20%:												
1 pt.	12	0	2	86	211	77	59	1	8	193	291	150
1 qt.	15	0	0	11	264	111	63	0	2	73	223	257
2 qt.	10	0	0	10	182	70	52	0	2	23	190	209
Kelthane WP, 25%:												
1 lb.	14	0	2	261	252	73	42	1	18	405	260	171
2 lb.	17	0	1	73	183	120	89	0	9	208	226	218
4 lb.	15	0	0	27	281	122	73	0	4	73	286	218
Aramite WP, 25%, 0.66 lb.	7	0	1	217	221	68	83	0	9	403	291	174
Oil emulsion, 90%, 3 qts.	6	0	1	177	221	125	61	0	3	315	278	288
L.S.D. at 5% level	ns	—	—	67	ns	ns	ns	—	17	148	ns	ns
at 1% level	—	—	—	89	—	—	—	—	22	196	—	—

[a]Prespray.

ganese sulfate plus ¼ pound of hydrated lime was substituted for manganese oxide when 1 pound of wettable Kelthane was used and 2¼ pounds of manganese sulfate plus 1¼ pounds of hydrated lime when 2 pounds of wettable Kelthane was used. Thorough coverage was accomplished. This post-bloom spray was applied between April 20 and May 5. On August 1 and October 31 all trees were dusted with sulfur for control of rust mites.

Records of infestation were taken at intervals throughout the season to obtain information on both the immediate effectiveness and persistence of the treatments. The active stages and unhatched eggs were counted by placing a hand lens with a 1-square-inch field, such as a linen tester, on the upper surface of 25 leaves per tree so that the midrib extended diagonally across the lens (Spencer & Selhime 1955). The results on the 10 trees receiving each treatment are shown in table 1.

The prespray counts varied from 6 to 17 crawling mites and from 42 to 89 unhatched eggs per 250 square inches, with no significant differences. The first post-spray counts indicated good immediate control of all stages. In June mite infestations were still low, but there were indications that they were beginning to increase.

By late July there were marked increases in infestation regardless of treatment, and yet they were significantly lower on trees that had been sprayed with 1 or 2 quarts of emulsifiable Kelthane or 2 or 4 pounds of wettable Kelthane than on those that received other treatments. By this time infestations in the Aramite-sprayed trees were higher than in the untreated checks, and there were no significant differences between the oil emulsion and the untreated checks. This indicated that the stronger sprays of Kelthane had a long-lasting effect.

By the middle of September counts for all treatments had increased to an even plateau, not high enough to justify respraying but showing no significant differences. By mid-November there was a natural decline and no significant difference between treatments. Respraying was not necessary at the end of the year.

On December 5, 12 fruits were cut from each Kelthane-sprayed tree and larger samples from the trees that had received no acaricide, and sent to the Rohm & Haas Company for residue analyses.[2] In the 7 months between spraying and fruit sampling 29.15 inches of rain had fallen. The fruits sprayed with the emulsions showed from 0 to 0.11 p.p.m. in surface extracts, 0 to 0.03 p.p.m. in pulp, and 0.27 to 0.46 p.p.m. in the peel. The average residues from the wettable-powder sprays were lower. All residues were very low, although the fact that any measurable residues were found after such a long interval indicates persistence of the material. In view of the experimental tolerance of 7 p.p.m. Kelthane on citrus there seems to be no danger of harmful residues if the material is used in the regular post-bloom spray. The finding of lower residues in the pulp than in the peel is also favorable, since the pulp and juice are the edible portions. Further experimental work is needed to determine residue levels when applications are made nearer to picking time, as a basis for the setting of formal tolerances.

There was no phytotoxicity or damage from any of the sprays.

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[2] C. B. Lyon, E. L. Stanley, and Charles F. Gordon made these analyses.

Control of Fleas in Yards[1]

H. G. Wilson, J. C. Keller, and Carroll N. Smith,[2]
Entomology Research Branch, Agr. Res. Serv., U.S.D.A.

The use of DDT in the control of fleas on dogs was reported first by Lindquist *et al.* (1944). They were able to free dogs of these pests and protect them from reinfestation for 4 to 7 days by thoroughly rubbing a 4% or 5% DDT powder into the hair. Smith & Burnett (1948) found that chlordane, BHC, dieldrin, and aldrin were more effective than DDT. These materials comprise the principal insecticides that have been used in recent years in practical control work. However, Kilpatrick & Fay (1952) reported that the cat flea was not controlled in infested premises after as many as three applications of 5% DDT powder, but that infestations were greatly reduced or eliminated with a 5% chlordane dust. The same investigators reported that when successive generations of the oriental rat flea, *Xenopsylla cheopis*

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[2] P. H. Adcock and C. R. Crittenden performed many of the operations connected with this research.

Table 1.—Effectiveness of nine insecticides for the control of natural populations of cat and dog fleas in yards, when applications were made at 1 gallon per 1,000 square feet.

Treatment	Per Cent Control After Days Indicated							
	1	7	14	21	28	42	49	63
Diazinon EC								
0.5%	100	99	100	99	98	99	99	99
1.0%	95	100	99	100	100	99	97	99
Lindane EC 1.0%	100	58	100	99	100	100	100	—
Malathion EC								
2.0%	99	99	99	—	99	24	—	—
2.0%[a]	78	94	83	—	55	—	—	—
1.0%	100	100	100	100	100	100	100	100
	100	100	100	100	100	100	100	100
	100	100	100	0	—	—	—	—
	97	88	61	—	—	—	—	—
Dow ET-57 EC 1%	100	90	90	94	100	100	100	100
WP 1%	91	98	97	56	—	—	—	—
	97	59	95	100	99	100	100	100
Chlorthion EC 1%	85	80	85	100	100	100	100	100
	100	100	100	100	100	100	100	100
Layer 21/199 EC 1%	94	78	100	100	100	100	100	100
Chlordane EC 1%	11	25[b]	—	—	—	—	—	—
	70	99	100	100	100	100	100	100
	64	58	94	96	98	98	100	100
	99	100	98	100	100	100	100	100
	—	100[c]	100	100	61	100	100	—
Am. Cyanamid 4124 EC 1%	100	94	97	94	100	100	100	100
	100	100	100	100	100	100	100	100
	94	0	—	—	—	—	—	—
	91[d]	0	—	—	—	—	—	—
Dipterex soln. 1%	100	94	96	98	100	100	100	100
	96	21	—	—	—	—	—	—
DDT EC 1%	86	99	45	—	—	—	—	—

[a] ½ gallon used per 1,000 square feet.
[b] Re-treated, see text.
[c] Second treatment, see text.
[d] Count taken 20 minutes after treatment.

(Rothsch.), were subjected to selection by exposure to DDT their resistance increased through the F_3 generation, but no further increase was shown through the F_7 generation. The degree of resistance was not considered sufficient to hinder control with DDT.

Recently numerous reports have been received from central Florida that cat and dog fleas (*Ctenocephalides felis* (Bouché) and *C. canis* (Curt.)) could not be controlled with repeated applications of DDT or chlordane in locations where these insecticides had been effective previously. A series of tests were run to evaluate DDT, chlordane, lindane, Diazinon, malathion, Chlorthion, Am. Cyanamid 4124, Dipterex, Bayer 21/199, and Dow ET-57 (sampled as Dow ET-14) on naturally infested premises in the vicinity of Orlando. These premises had already been treated by the owners one or more times with DDT or chlordane without obtaining relief. The soil in and around dog houses was frequently heavily infested and flea production was continuous. The adults migrated rapidly and created a nuisance over the entire yard. At times they even invaded the premises of neighbors. The dry, sandy areas under houses, which are favorite resting places for dogs during hot weather, were especially troublesome. Such areas are difficult to spray and often are treated only partially or not at all.

Methods.—Sprays of the various insecticides were applied to infested yards, dog pens and houses, and other areas that had become heavily infested. Most of the treated premises were about 5,000 square feet in area, but a few ranged from 1,200 to 10,000 square feet. Applications were made with a power sprayer operating at 150 p.s.i., usually at a rate of 1 gallon per 1,000 square feet. An orchard-type nozzle capable of projecting a stream at least 25 feet assured reaching inaccessible areas under the houses.

Estimates of flea abundance were made by counting those landing or crawling in 1 minute on the lower parts of the legs of the observer. Counts were made at eight locations in each yard before and at various intervals after treatment.

Results.—The results of these tests are summarized in table 1. All the insecticides except chlordane and DDT were generally fast-acting and highly effective, and all except DDT gave 99 to 100% control for 7 to 9 weeks in some or all of the tests.

At equal application rates a spray containing 2% of malathion was no more effective than a 1% spray. When the amount applied was reduced to ½ gallon per 1,000 square feet, 2% malathion gave much less control than most of the 1-gallon applications of 1% malathion. Chlordane was slow-acting in some yards, but eventually caused 100% mortality in five. It was unsatisfactory in one yard, but a second treatment gave complete control. DDT failed to give satisfactory control.

Reinfestation by newly emerged fleas decreased the effectiveness of some of the treatments for short periods in some areas where infestations had become well established. However, a high degree of control was re-established where the insecticide had a long residual action. Since most of the insecticides gave satisfactory reductions, the control problem does not appear to be acute in central Florida. Faulty application was probably responsible for some of the reported failures. Special attention should be paid to obtaining a thorough coverage with a sufficient quantity of insecticide to kill the fleas. A suggested application consists of 1 gallon of a 1% spray for each 1,000 square feet. A coarse nozzle that will prevent too extensive atomization should be used, and the spraying pressure should be sufficiently high to assure reaching all the areas needing treatment.

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Effect of Spray Date on Residues of Chlorinated Hydrocarbon Insecticides on Peaches[1]

Jack E. Fahey, D. W. Hamilton, and Harold W. Rusk,
Entomology Research Division, Agric. Res. Serv., U.S.D.A.

Existing tolerances for residues of chlorinated hydrocarbon insecticides on peaches include 7 p.p.m. of DDT, 0.3 p.p.m. of chlordane, and 0.1 p.p.m. of dieldrin. There are no established tolerances for heptachlor, a component of chlordane, or endrin, the stereoisomer of dieldrin, although these materials might very well be used as substitutes for chlordane or dieldrin. DDT is often recommended for control of the oriental fruit moth, *Grapholitha molesta* (Busck), in peaches, and dieldrin and chlordane have been recommended for the control of the plum curculio, *Conotrachelus nenuphar* (Hbst.), and insects that catface peaches. Late broods of both insects may be expected to attack peaches during July and August in southern Indiana and Illinois.

Because of the need of insecticides for control of these pests, an

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Table 1.—**Insecticide residues on peaches, 1956. Parts per million.**

Subplot	Days Final Spray to Harvest	After Final Spray	Half-Way to Harvest	At Harvest
		DDT		
1	40	40.8	21.8	5.3
2	30	53.6	24.5	11.9
3	20	52.3	24.3	17.4
4	10	42.1	31.1	24.6
		Chlordane		
1	40	—	4.9	0.8
2	30	9.3	5.0	1.1
3	20	21.0	6.7	2.0
4	10	10.6	7.8	2.2
		Heptachlor		
1	40	—	0.2	0.03
2	30	5.0	.5	.05
3	20	3.8	.6	.12
4	10	5.0	.9	.36
		Dieldrin (Organic Chlorine)		
1	40	3.8	0.9	0.3
2	30	6.4	1.5	.8
3	20	6.3	1.7	.9
4	10	3.2	1.5	1.3
		Endrin (Organic Chlorine)		
1	40	0.6	0.07	0.08
2	30	3.0	.8	.3
3	20	1.6	.5	.4
4	10	1.4	.4	.7

experiment was conducted to determine the chlorinated hydrocarbon residues that would remain in peaches following late-season sprays.

The orchard area used in this experiment was isolated from other orchards to avoid contamination. Each plot consisted of 12 trees and was divided into four subplots to permit variations in date of final application. The sprays were applied with a conventional hydraulic sprayer and single-orifice spray guns. Prebloom applications of sprays or dusts had been made uniformly over the entire area, but beginning with petal fall each plot was treated with a different insecticide according to the schedule below. The toxicants used were 50% DDT at 2 pounds, 50% dieldrin at 1 pound, 19.5% endrin at 10 ounces, 25% heptachlor, at 1 pound, and 40% chlordane at 3 pounds per 100 gallons.

Date	*Spray*	*Material Per 100 Gallons*
April 27	Petal fall	All toxicants plus wettable sulfur 5 lbs.
May 8	Shuck-off	All toxicants plus wettable sulfur 4 lbs.
16	First cover	DDT plus wettable sulfur 4 lbs.; dieldrin, endrin, heptachlor, and chlordane plus parathion 1.5 lbs. and wettable sulfur 4 lbs.
24	Second cover	Same as first cover.
June 14	Third cover	All toxicants plus parathion 1.5 lbs. and captan 1 lb.
July 7	Fourth cover	DDT, heptachlor, and chlordane plus parathion 1.5 lbs. and captan 1.5 lbs.; dieldrin and endrin plus parathion 1.5 lbs. and wettable sulfur 4 lbs.
17	Fifth cover (subplots 2, 3, and 4 only)	Same as fourth cover.
27	Sixth cover (subplots 3 and 4 only)	Same as fourth cover.
Aug. 6	Seventh cover (subplot 4 only)	Same as fourth cover.

Samples of 80 peaches were collected from each subplot immediately following the final cover spray, half-way between the final spray application and harvest, and at harvest (August 16). These samples were divided into four 20-fruit replicates and analyzed for residues. DDT residues were determined by the Stiff-Castillo (1945) method, heptachlor and chlordane by colorimetric methods employing the Davidow (1950) reagent, and dieldrin and endrin were analyzed for organic chlorine by the Phillips-De Benedictis (1954) method.

The results of analysis of four replicates from each subplot have been averaged to obtain the data shown in table 1. Rainfall during the period of study was above normal. At Vincennes (about 5 miles from the orchard) 6.3, 4.5, 3.1, and 1.7 inches were recorded the last 40, 30, 20, and 10 days before harvest, respectively.

The disappearance curve of data from the subplots treated 40 and 30 days before harvest (extrapolated) shows the DDT residue to reach 7 p.p.m. 37 days after the final spray. The initial samples from chlordane and heptachlor subplots treated 40 days before harvest were lost. The data indicate that chlordane residues from sprays applied 40 days before harvest would exceed the tolerance of 0.3 p.p.m. Heptachlor residues from sprays applied 20 days before harvest averaged only 0.12 p.p.m.

The organic chlorine residues are not a precise measure of the dieldrin or endrin residues, since they could be from degraded dieldrin or endrin. They do indicate that applications within 30 days of harvest could result in residues in excess of 0.1 p.p.m.

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The Overwintering Habitat of the Sugar-Pine Cone Beetle[1]

Herbert Ruckes, Jr., *University of California, Berkeley*

The habit of twig mining by the sugar-pine cone beetle, *Conophthorus lambertianae* Hopk. (Coleoptera: Scolytidae), had been observed and reported by Struble (1947). This same twig mining habit was again observed in 1956 in several areas in California.

In May, 1956, Dr. R. C. Hall[2] made a collection of mined twigs of sugar pine from Alamo Mountain, Los Angeles County. Later the same month, the author made collections of mined twigs from Pinecrest, Tuolumne County and from an area north of Upper Lake, Lake County. Each of these mined tips contained an adult cone beetle.

In the first week of September, 1956, newly mined twigs became evident on the branches of sugar pines in the Sierra Nevada Mountains. The mined tips are quite obvious by the silvery color of the needles early in the fall which later turn to straw yellow. Twigs mined during previous seasons can be distinguished by the dark red-brown color of the needles. Upon examination of these twigs, mature cone beetles were found burrowing in the tips. The beetle enters the twig tip at the base of the vegetative bud and mines only the current year's growth, remaining at the

[1] Studies of the cone and seed insect problems in the California pine region made possible by a grant from the Gilbert M. Walker Trust. Accepted for publication March 1, 1957.

[2] Forest Entomologist, California Range and Forest Experiment Station, U. S. Dept. of Agriculture, Berkeley, California.

end of the burrow with its posterior toward the distal or bud end of the twig. Periodic collections and examinations following this first observation disclosed that the beetles were in the twigs during the winter and early spring of 1957.

The twig mining and overwintering habit of the red-pine cone beetle, *C. resinosae* Hopk., has been reported by Packard (1890) and Lyons (1956).

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Insect Mortality at Low Temperatures[1]

Edward B. Knipling, *Plant Pest Control Branch*, and
W. N. Sullivan, *Entomology Research Branch*,
Agric. Res. Serv., U.S.D.A.

The potential spread of destructive insects through hitchhiking on aircraft is causing increasing concern to quarantine entomologists. During recent years much research has been conducted to develop suitable disinsectization treatments. The coming of the jet age has perhaps brought an answer to some of the problems of quarantine treatment on jet-powered aircraft. For efficient operation jet aircraft must fly in the stratosphere at 40,000 feet, where outside temperatures range from −42° to −65° C. (−43.6° to −85° F.). On sustained flights unheated parts of jet aircraft where insects hide may reach temperatures that approach these outside ranges, and thus kill all or most of the insects that may be transported in such aircraft.

In order to obtain information regarding this possibility, low-temperature studies were conducted in the laboratory to determine the mortality points of several insects (see also Wigglesworth 1950, Packchanian 1954).

The test insects were placed in wire-screen cages 9¼ inches in length and 3¼ inches in diameter. A nearly constant number of each species of laboratory-reared insects was used for each test; mosquitoes averaged approximately 100 per test, and others 15 to 30. The number of field-collected insects per test varied because of difficulty in obtaining the desired number of some of them.

Two 6.5-cubic-foot cold cabinets capable of reaching −65° C. were used to simulate high-altitude temperatures. An 18-cubic-foot home freezer capable of reaching −20° was also used.

The first series of tests were made to determine the degree of cold necessary to kill the insects when exposed for 60 minutes. This was done by holding the insects at temperatures ranging from 0° to −30° C. Control dials were on each freezer, and the freezers were adjusted to the desired temperature 24 hours before a test was begun. A thermometer was kept in each freezer at all times and was often used for checking accuracy. There was often a variation of 1 or 2 degrees within the test period, which was due to the operation of the thermostatic unit. At the end of each test period the immediate knockdown was recorded. The insects were fed and kept in an outdoor insectary for a 24-hour observation period where temperatures ranged from 10° to 35° C. (50° to 95° F.). Approximately 40 tests were conducted in this manner.

The mortalities of all test insects after 60-minute exposures are given in table 1. The data are grouped in 5-degree increments, each having a tolerance of ±2 degrees. Each mortality represents the mean of four tests. The standard error was calculated for this mean and is also given.

Mortalities less than 100% have minor importance in relation to the object of the basic work, which was to find the temperature that causes 100% mortality of each insect.

The point of 100% mortality of the American cockroach, Mexican bean beetle larva, Mexican bean beetle adult, house fly, grasshoppers, and Japanese beetle was found to be −15° C. The confused flour beetle and the American dog tick had 100% mortality at this temperature, but they also had an unexplained low survival at −20°. In one out of seven tests at the lower temper-

[1] Accepted for publication March 7, 1957.

Table 1.—Percentage mortality in 24 hours of several insects held for 1 hour at low temperatures.[a]

Species	Stage	Temperatures Used					
		0° C.	−5° C.	−10° C.	−15° C.	−20° C.	−30° C.
Yellow-fever mosquito (*Aedes aegypti* (L.))	Adult	62± 8	99± 1	100	—	100	100
American cockroach (*Periplaneta americana* (L.))	Nymph and adult	2± 2	71±10	98± 2	100	100	100
Mexican bean beetle (*Epilachna varivestis* Muls.)	Larva	10± 4	50±16	94± 4	100	100	—
	Adult	6± 3	36± 5	90± 7	100	100	—
House fly (*Musca domestica* L.)	Adult	4± 3	41±13	88±12	100	100	100
Grasshoppers[b]	Nymph and adult	7± 2	33±11	87±10	100	100	100
Japanese beetle (*Popillia japonica* Newm.)	Adult	0	42± 2	84±12	100	100	100
Confused flour beetle (*Tribolium confusum* Duv.)	Adult	14±10	32±16	81±11	100	90±10	100
American dog tick (*Dermacentor variabilis* (Say))	Adult	0	26±11	48±15	100	88±12	100

[a] The flies, mosquitoes, cockroaches, and flour beetles were reared at 27° C.; the other insects were field-collected during June and July at Beltsville, Md.

[b] *Dichromorpha viridis* Scud., *Eritettix simplex* Scud., *Syrbula admirabilis* Uhler, *Arphia sulphurea* Fab., *Chortophaga viridifasciata* (DeG.), *Hippiscus rugosus* Scud., *Dissosteira carolina* (L.), *Schistocerca americana* (Drury), *Melanoplus bivittatus* (Say), *Melanoplus mexicanus* (Sauss.).

Table 2.—Percentage mortality in 24 hours of adult house flies and adult mosquitoes after being held for various periods at −20° C.

MINUTES EXPOSURE	HOUSE FLIES	YELLOW-FEVER MOSQUITOES
1	12	56
2	40	96
3	41	97
6	79	100
9	94	99.5
12	97	100
15	99	100
30	100	100
45	100	100
60	100	100

ature the flour beetle had 5 survivors out of 175 insects tested, and the dog tick had 5 survivors out of 72 insects tested. In the other six tests there were no survivors. The mosquito proved to be comparatively easy insect to kill. Complete mortality was obtained at −10°. All the species showed 100% kill at −30°.

In the second series of tests yellow-fever mosquitoes and house flies were held at a constant temperature of −20° C. for various periods to determine the minimum time necessary for complete mortality.

Table 2 gives the percentage mortality of adult yellow fever mosquitoes and house flies after being held for various periods at −20° C. A 6-minute exposure gave 100% kill of the mosquitoes, 2 out of 400 survived at 9 minutes, and 12 minutes and longer exposure gave 100% mortality in all tests. Complete mortality of the house fly came after 30-minute exposures, whereas the 15-minute exposures averaged 99% kill. The immediate knockdown of all insects except the American dog tick was consistently 100%.

These results strongly indicate that many insects of agricultural and public health importance would be killed if exposed for 1 hour to temperatures between −15° and −30° C., and the house fly and yellow-fever mosquito if exposed for 30 minutes or longer to −20°.

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Carrot Beetle Control in Domesticated Sunflowers[1]

C. C. BURKHARDT,[2] *Kansas State College, Manhattan*

The carrot beetle, *Ligyrus gibbosus* (DeG.),[3] was extremely abundant in Riley County, Kansas, during mid-August, 1956. David L. Matthew, survey entomologist, collected more than 22,000 beetles in a light trap at the College Agronomy Farm during the night of August 16. Highest catches were obtained from August 13 to 18.

One of the beetle's favorite host plants is the common sunflower, *Helianthus annuus* L. Several varieties which harbored as many as 90 beetles per plant were grown in test plots on the Agronomy Farm. Such large numbers of beetles feeding on the roots and base of the stalks of the sunflowers resulted in severe damage, consisting of lodging and failure to produce seed.

MATERIALS AND METHODS.—Chemical control tests were initiated August 16. Emulsifiable concentrates were added to water and applied at the rate of 25 gallons of emulsion per acre,

Table 1.—Effectiveness of dieldrin and heptachlor applied as emulsion sprays and granulated formulations against the carrot beetle in sunflowers at Manhattan, Kansas, on August 18, 1956.

POUNDS TOXICANT PER ACRE	AVERAGE NUMBER BEETLES PER PLANT	AVERAGE NUMBER		PER CENT DEAD	CORRECTED[a] PER CENT MORTALITY
		ALIVE	DEAD		
Emulsions					
Heptachlor, 5	42	1	41	97.6	97.3
Dieldrin, 3	35	3	32	91.4	90.4
Granules					
Heptachlor, 7.5	36	2	34	94.4	93.7
Dieldrin, 4.5	28	3	25	89.2	88
Check	39	35	4	10.2[b]	—

[a] Based upon 10.2% dead, using Abbott's formula as given by Abbott (1925).
[b] Mortality in check could have resulted from natural causes but, more likely, by poisoned beetles having crawled into the check plots before dying.

dircteed toward the base of stalks and on the ground for at least 6 inches on both sides of the row. The granulated formulations were applied by hand as a 6-inch band down the row. The check plots were adequately protected from drift of chemicals from the treated plots.

Five to ten samples were taken in each plot August 18, two days following insecticide application. On that date, numerous dead beetles were lying on the surface of the soil down the treated rows, but not down the untreated rows. Each sample was taken by counting the number of live and dead beetles in a 6-inch radius both on the surface and 2 inches deep in the soil around the base of each plant.

RESULTS.—Table 1 shows that both dieldrin and heptachlor gave economic control in both formulations. Heptachlor appeared to act more rapidly than dieldrin.

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[1] Contribution No. 677, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan. Accepted for publication March 13, 1957.
[2] Assistant Entomologist, Kansas Agricultural Experiment Station.
[3] Order Coleoptera, Family Scarabaeidae.

The Lily Bulb Thrips on Easter Lily

CHARLES F. DOUCETTE, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

Liothrips vaneeckei Pries., the lily bulb thrips, is recognized as a pest of certain species and varieties of lilies (Schopp & Doucette 1932, Bailey 1939). It is most troublesome to those types with loosely scaled bulbs. The bulb of the Easter lily (*Lilium longiflorum*) is fairly compact and has overlapping scales with little space between them where the bulb thrips could feed.

Many more bulbs of this lily are being grown in the United States than of any other type, but it was not until 1952 that a serious infestation by lily bulb thrips was observed. Then plants of the varieties Croft and Ace in a western Washington home garden were found infested at digging time. The populations were moderate, and both adults and larvae were present on the subterranean stems as well as in the bulbs. Approximately as many individuals were on the stems as in the bulbs. In the bulbs most of them were in the central area around the stem. The

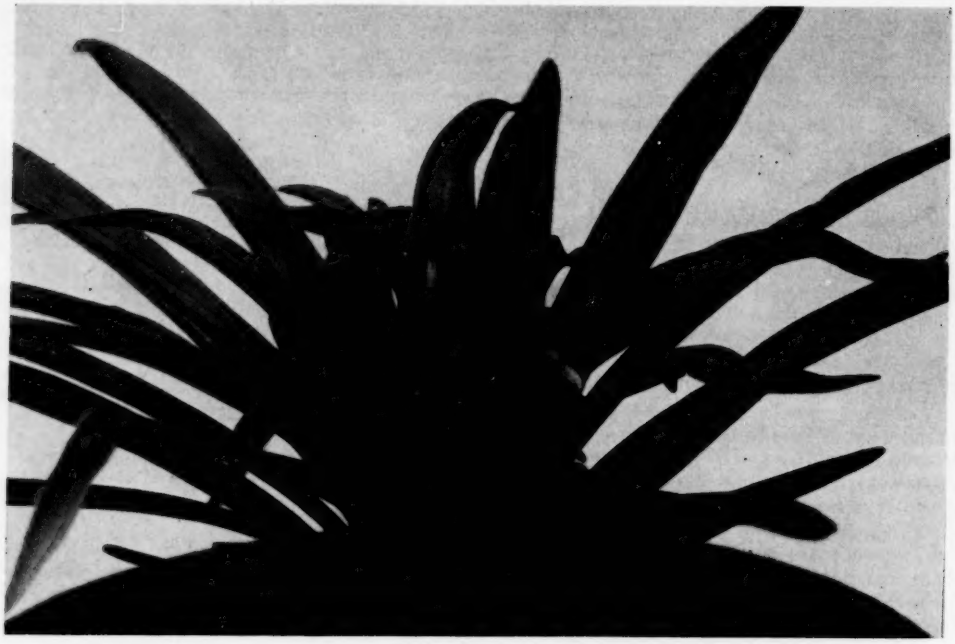

FIG. 1.—Distortion of foliage of an Easter lily caused by feeding of lily bulb thrips during early development of the shoot.

others were between looser scales, wherever these happened to be. It was evident that the thrips selected locations where the bulb parts did not press together too closely.

Damage to Easter lilies in an Oregon greenhouse was reported by Bock in 1955. It appeared that this infestation was carried with the bulbs from the commercial producer. Only about 250 of the 10,000 bulbs forced in the greenhouse were infested. The thrips were killed by spraying with parathion, but 50 of the plants were damaged so severely that they were discarded.

In the 1956 season damage was observed in four greenhouses in western Oregon and western Washington. Less than 1% of the plants in the greenhouse were infested. A study of the attack indicated that the thrips injured the tips of the young shoots as they grew out of the bulbs. The feeding caused considerable distortion of the early leaves (fig. 1) and occasional slight stem distortion. A characteristic indication of the injury was a sharp hook at the leaf tip. Where injury was noted in the early growth of the plants, treatment with insecticides allowed most of the plants to grow to suitable salable condition.

Apparently only a very few thrips on the bulbs can initiate a colony sufficient to do damage under greenhouse conditions. After potting the bulbs the greenhouse operator keeps them at a moderate temperature, 45° to 50° F., to permit rooting. The thrips feed on the new shoots as they slowly grow from the bulbs. Infestation is not realized until there is a moderate amount of growth above ground.

In a western Washington establishment an entirely new type of injury occurred. Adult thrips from infested plants in one house spread to lilies in an adjacent unit when the plants were in the small bud stage. The buds were then hardly ½ inch long and were in a tight cluster loosely enclosed by young leaves 1 to 2 inches long. The thrips went into the bud clusters, where they found conditions favorable for reproduction and development. Small colonies of up to 15 to 20 individuals developed in these clusters, and their feeding on the buds and stems distorted the leaves and flowers (fig. 2). The cause of this injury was not recognized until considerable damage had been done. Over 50 plants of 2000 in this house were valueless because of the distorted flowers.

FIG. 2.—Distortion of flowers of an Easter lily resulting from feeding of lily bulb thrips in bud cluster during early bud stage.

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An Apparatus for Measuring Corn Earworm Injury to Sweet Corn[1]

L. P. DITMAN and J. L. DITMAN[2]

A device for measuring insect injury to sweet corn was described by Connell (1956). At the Maryland Station we have been using for several years a similar but slightly more complicated measuring apparatus (fig. 1) which may be used to measure the length of the ear in inches, the depth penetration in inches or in percentage of ear length, and the actual per cent of loss caused by the insects. For measuring the actual loss the machine must be calibrated for each variety of corn because the shape and distribution of kernels along the ear may differ with variety.

The apparatus is constructed of six pieces of wood, a piece of heavy clock spring, and a small piece of sheet metal which is used for the index arm point and guide. The base is made from ¼-inch plywood 26×14 inches. The sides of the angle are made of ¾-inch lumber 25¾×2½ inches. The index arm is ¼-inch wood ¾ by 25 inches and is attached to the piece forming the upper side of the angle by a single screw so that the lower edge passes through the vertex of the angle. Washers are placed on the screw on top and bottom to permit it to pivot freely. A small piece of sheet metal is fashioned to form the index arm point, which is extended over the scale and is in line with the lower edge of the index arm. The metal forming the index point may be shaped so that a portion of it may be bent around the end of the index arm so as to extend under the scale to form a guide, or a second small piece of sheet metal may be used for lower index arm guide. One end of a strong piece of clock spring about 3 inches long is attached to a block near the vertex of the angle, the other end rests against the index arm to return it to the zero position after each measurement. The scale is made of ¼-inch plywood; it is curved to correspond to the arc of the index arm and is attached to the open ends of the side pieces forming the angle.

Table 1.—Conversion of per cent length of ear penetrated to per cent actual loss of grain of Asgrow Bantam Evergreen corn, based on 10 typical ears of varying length.

PER CENT OF EAR PENETRATED	PER CENT OF GRAIN LOST
10	2.2
20	8.8
30	18.7
40	30.3
50	42.7
60	56.0
70	69.6
80	82.4
90	93.8
100	100.0

The base is calibrated for measuring the ear in inches by drawing lines perpendicular to a line bisecting the angle at the places where the side arms are 4, 5, 6, and up to 12 inches apart. The scale is calibrated for per cent depth by reference to the 10-inch length line where each inch equals 10 per cent. A ruler held across the sides of the angle just under the index arm at the 10-inch line will facilitate marking the scale.

For measuring the depth of penetration in inches the ears of corn are held on the 10-inch base line with the tip of the ear against the upper side of the angle (the point where the index arm reads zero on the scale); the index arm is then moved down to where the lower edge corresponds to the point of deepest insect

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[2] L. P. Ditman, Department of Entomology, University of Maryland; J. L. Ditman, Senior, College of Engineering, University of Maryland. The apparatus was designed and constructed by the second author.

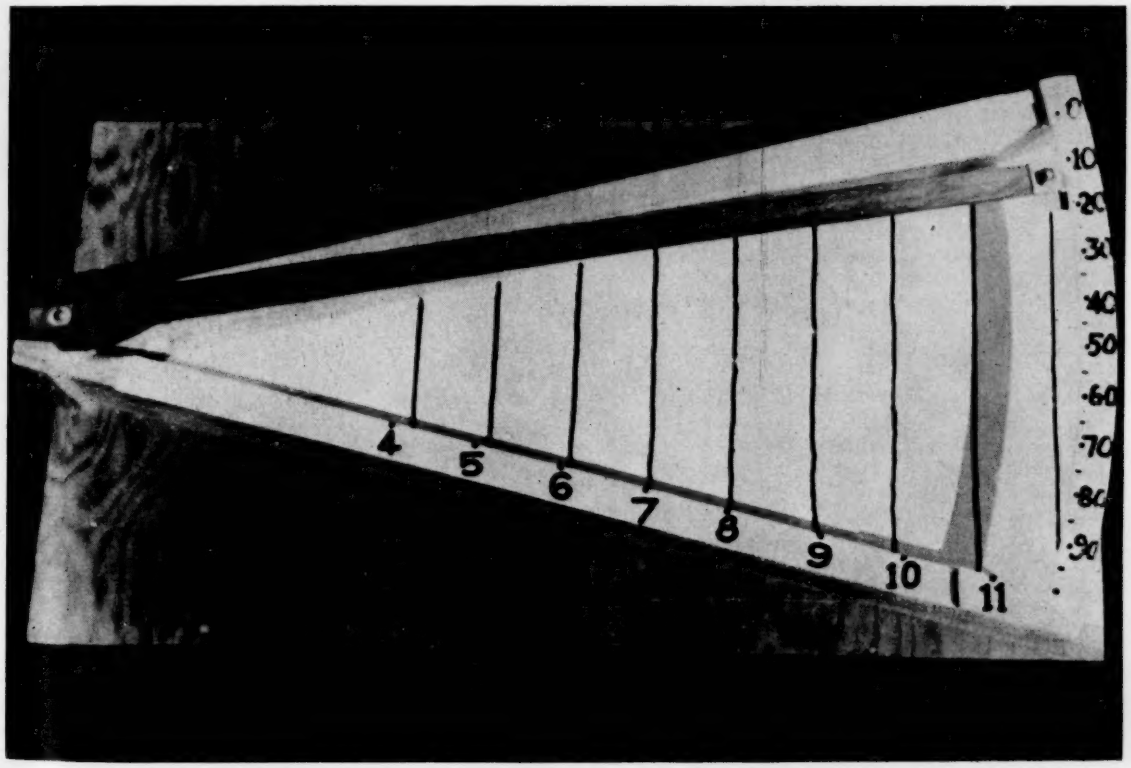

FIG. 1.—Apparatus for measuring insect injury to sweet corn.

penetration; the reading on the scale is inches penetrated. For measuring length of ear and per cent of ear penetrated the ear is held parallel to the base lines at the place where the tip of the ear touches the upper side of the angle and the butt rests against the lower side of the angle; the index arm is moved down to the point of deepest penetration; the reading on the base is length in inches and the reading on the scale is percentage penetrated.

To determine actual per cent loss, grains of corn are removed from typical uninjured ears of a variety at 10%-intervals and weighed. This can be most easily done by drying the corn before removing kernels. From this information a conversion table was computed (table 1); an additional scale may be constructed to read in actual per cent loss of ear. The actual per cent loss is particularly applicable to canning sweet corn where entire tips of ears are cut off in the trimming operation.

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Leafhoppers Infesting Lawns in Rhode Island[1]

T. W. KERR,[2] *University of Rhode Island, Kingston*

Although it is generally known that leafhoppers are a problem in some lawns, little information is available relative to the species involved and their turfgrass preferences. Accordingly, a survey of leafhoppers inhabiting lawns in Rhode Island was conducted during the season of 1956. Fifteen lawns containing the major species of turfgrass grown in the state were examined. Beginning in late May and continuing until late October some of these were included in a periodic survey, others were sampled as opportunity permitted. Some harbored relatively few leafhoppers, while at the same time other similar areas were heavily infested. In Rhode Island many lawns have been seeded with mixtures containing Kentucky bluegrass, *Poa pratensis* L., Chewings fescue, *Festuca rubra commutata* Gaud., and Colonial bentgrass, *Agrostis tenuis* Sibth. Annual ryegrass. *Lolium multiflorum* Lam., is often included in the seed mixture. As a result, the latter is often a component grass species, at least for the first season.

Of a total of 35,576 leafhoppers collected in 7,700 sweeps with a 12-inch net, 44.7% were *Macrosteles fascifrons* (Stal.), 19.9% *Deltocephalus pulicaris* (Fall.), 13.5% *Endria inimica* (Say), 4.7% *Graminella nigrifrons* (Forbes) and 2% comprised *Helochara communis* Fitch, *Aceratagalia sanguinolenta* (Prov.), *Agallia constricta* VanD., *Draeculacephala antica* (Walk.), *Empoasca fabae* (Harr.), *Paraphlepsius irroratus* (Say) and *Cloanthanus frontalis* (VanD). Immature leafhoppers accounted for 15.2% of the total. The majority of these were among the four most abundant species listed above.

Another insect found throughout the collection period in all lawns was the frit fly, *Oscinella frit* (L.). In number it exceeded the total for leafhoppers and was especially abundant during late June and early July.

Macrosteles fascifrons was particularly abundant where annual ryegrass was present. It was collected in slightly smaller numbers from turf areas consisting of Kentucky bluegrass and Chewings fescue. *Deltocephalus pulicaris* showed a definite preference for Chewings fescue. It was collected also from Kentucky bluegrass but not from annual ryegrass. Although *Endria inimica* showed a preference for Chewings fescue, it was found also in lawns containing Kentucky bluegrass and Colonial bentgrass. It was equally abundant in lawns that were relatively weed-free and lawns where weeds abounded. Beirne (1956) has mentioned that heavy infestations of this species sometimes develop on lawns in southern Ontario. *Graminella nigrifrons* also showed a preference for Chewings fescue but was present where other grass species were grown. In unpublished studies of turf leafhoppers in Florida, F. W. Mead found the latter species among those collected in abundance from several grasses of importance in that state.

Populations of *M. fascifrons* were greatest during early June, mid-July and again in late August; those of *D. pulicaris* occurred in late June and late August. *E. inimica* was most abundant during early July and early September, while *G. nigrifrons* had periods of abundance during late June, late August and early October.

A single spray application containing 1.2 ounces of actual DDT in 7.5 gallons of water per 1000 square feet of turf effectively reduced populations of all species for periods as long as 2 months. The same amount of methoxychlor performed similarly against *G. nigrifrons* and *D. pulicaris*, but provided control of *M. fascifrons* and *E. inimica* for only half as long.

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[1] Contribution No. *916* from the Rhode Island Agricultural Experiment Station, Kingston, Rhode Island. Accepted for publication March 22, 1957.

[2] The author gratefully acknowledges the assistance of D. A. Young in establishing the identity and taxonomic position of the leafhopper species mentioned herein. The author is also indebted to F. W. Mead, Entomologist of the State Plant Board of Florida, for permission to cite certain of his unpublished data on leafhoppers from turf in that state.

Laboratory Evaluation of Contact Insecticides on Three Species of Stored-Product Insects[1]

L. E. VINCENT and D. L. LINDGREN, *University of California Citrus Experiment Station, Riverside*

Stored-product insects can be controlled either by fumigation or by the use of contact insecticides. Various contact insecticides are used to impregnate sacking or packaging materials, or are incorporated with grain as a protectant, or are applied as sprays, dust, aerosols, etc., in sanitation programs. Laboratory evaluation of various contact insecticides on several species of stored-product insects was undertaken to determine possible usefulness in a control program.

Several methods of testing the effectiveness of contact insecticides against stored-product insects were tried. The insects were confined to walking on insecticide-treated surfaces of glass or filter papers, or they were given topical applications of the insecticides. Adults of 11 species of stored-product insects were confined on the treated surfaces of filter papers or glass, but it was impossible to obtain consistent results, as there seemed to be no constant relation between dosage and mortality. This was apparently due to the unevennesss of the deposit on the glass or filter paper, to the erratic movement of the insects over the deposit, and to the fact that the exposure time was limited to overcome any fumigation effects. In general, large increases in dosages were required to show small increases in mortality. Similar results were obtained with this method of evaluation by Busvine & Nash (1953).

The topical method appeared to be the most reliable of those tested, although it is restricted to measurement of initial toxicity of insecticides and does not take into consideration prolonged residual effects. With this technique, small increments in dosages gave significant increases in mortality, and the results of replications were relatively consistent.

The method used was similar to that described by March & Metcalf (1949). Test insects included for comparison were fourth-instar larvae of the khapra beetle, *Trogoderma granarium* Everts; larvae and adults of the confused flour beetle, *Tribolium confusum* Duv.; and adults of the granary weevil, *Sitophilus gra-*

[1] Paper No. 944, University of California Citrus Experiment Station, Riverside California. Accepted for publication March 27, 1957.

Table 1.—**Micrograms of insecticide per insect to give 50 and 95% kill of khapra-beetle larvae, confused-flour-beetle adults and larvae, and granary-weevil adults.**

Insecticide	Khapra-Beetle Larvae		Confused-Flour-Beetle				Granary-Weevil Adults	
			Larvae		Adults			
	LD-50	LD-95	LD-50	LD-95	LD-50	LD-95	LD-50	LD-95
Methyl parathion	< 0.5	1.4	0.05	0.2	< 0.01	0.03	< 0.01	0.01
Parathion	< 0.5	2.7	0.12	0.7	0.01	0.02	0.01	0.03
Malathion	0.9	3.6	2.25	1.6	0.4	1.1	0.07	0.1
Pyrethrins[a]	0.9	4.2	0.36	1.9	0.09	0.4	0.2	0.8
Am. Cyanamid 4124	< 0.5	2.2	0.8	3.6	0.21	0.7	0.06	1.3
Chlorthion	1.9	8.8	0.58	2.3	0.18	0.4	0.2	0.4
Dieldrin	6.0	>25.0	1.0	3.6	0.17	0.5	0.02	0.07
Lindane	25.0	>25.0	1.0	4.6	0.17	0.8	0.06	0.2
Aldrin	6.5	>25.0	2.0	6.5	0.23	1.2	0.02	0.06
Heptachlor	13.0	>25.0	1.8	8.0	0.1	1.3	0.09	0.2
Allethrin	7.0	>25.0	3.0	15.0	0.27	0.9	2.3	8.4
Toxaphene	>25.0	>25.0	7.8	21.0	0.8	4.5	0.8	1.7
DDT	>25.0	>25.0	20.0	>25.0	0.19	0.4	0.8	2.5
Strobane	>25.0	>25.0	13.5	>25.0	2.3	8.0	1.3	1.9
Phostex	5.0	>25.0	17.0	>25.0	8.3	>25.0	10.2	22.0
Piperonyl butoxide	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	10.0	23.5
Chlordane	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	19.0	>25.0
Methoxychlor	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0
TDE	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>15.0	>25.0
Nicotine	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	23.0	>25.0

[a] Pyrocide 175 (20% pyrethrins).

narius (L.). Twenty-five insects were used for each test. Five to eight concentrations and from three to six replications of each concentration were used for each insecticide to establish a dosage-mortality curve.

The insecticides were dissolved in acetone and applied dorsally behind the head capsule. Parallel control experiments, using acetone only, were conducted and, although the mortality in the controls was low, results were corrected by the use of Abbott's formula (1925). Following treatment, the test insects were held with small amounts of food in petri dishes at a temperature of 80° F. and at 60% relative humidity. Mortality counts were made at the end of 5 days for confused-flour-beetle adults, at the end of 10 days for confused-flour-beetle larvae and granary-weevil adults, and at the end of 20 days for khapra-beetle larvae. Any larvae that pupated were considered alive. Results are shown in table 1.

Under the conditions of these tests, the following conclusions were reached:

1. In general, the organic phosphate insecticides (with the exception of Phostex) and pyrethrins were the most toxic of the insecticides to all stages of the three insect species tested.

2. The chlorinated hydrocarbons were relatively ineffective against khapra-beetle larvae.

3. Where the LD_{95} was less than 25 μg. per insect and comparisons could be made, it was observed that (a) khapra-beetle larvae were more resistant than the other species tested, except to Am. Cyanamid 4124; (b) the larvae of the confused flour beetle were more resistant than the adults; and (c) to 11 insecticides granary-weevil adults were more susceptible than confused-flour-beetle adults, and to four insecticides confused-flour-beetle adults were more susceptible than granary-weevil adults.

4. TDE, methoxychlor, chlordane, nicotine, Phostex, and piperonyl butoxide were the least toxic to all insects tested.

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Biology of *Solenotus begini* (Ashmead)[1]

Richard L. Doutt, *Department of Biological Control, University of California, Berkeley*

Solenotus begini (Ash.)[2] is an important parasite of the dipterous leaf-miners in California. Its known host range includes 19 species in 5 genera of Agromyzidae, and it is commonly found attacking such pests as *Liriomyza* spp. in commercially grown melons, lettuce, peas, spinach, and alfalfa.

The life history of *Solenotus begini* was first outlined by Webster & Parks (1913), but additional studies of this insect have shown the need for further description. These later observations were made on *S. begini* as a parasite of *Phytomyza atricornis* Meigen mining the leaves of *Sonchus oleraceus* L. held in the laboratory under the nearly constant conditions of 78° F. and 65% relative humidity. The stock of both hosts and parasites was obtained at Albany, California.

Solenotus begini is arrhenotokous. The female, whether mated or not, is quickly responsive to the host's mine which she detects as she walks across the leaf surface. The reaction to the mine is displayed irrespective of the presence of a host susceptible to attack. When the serpentine mine is encountered the female parasite progresses along its length, and as she does so she continually swings her body from side to side in order that her sensitive antennae can trace the margins of the mine. If the female reaches the terminus of the mine where the host maggot is feeding she normally unsheathes her ovipositor and stings the host. Stinging and oviposition are two separate and distinct procedures, for oviposition of the single egg into the mine occurs after stinging and at a few millimeters distant from the stung host. There is a delayed effect of the venom on the host maggot,

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[2] (Chalcidoidea, Eulophidae)

FIG. 1.—Top of leaf mine removed to show pupa of *Solenotus begini* (Ashmead) lying between pillars of meconium. (Photographed by F. E. Skinner)

which continues to feed after having been stung. However, in about 25 minutes the host's movements suddenly stop and death apparently occurs at that time.

The parasite egg hatches within 24 hours in the moist environment of the mine. Under drier conditions its hatching is much delayed, and may even be prevented.

The first instar larva is very motile and its progressive movement through the mine towards the host is aided by the numerous setae arranged along the body. Feeding begins immediately upon contact with the host and the parasite larva soon moults. As with many other external parasites, the feeding period is quite abbreviated and the complete larval period totals just 3 days.

When feeding is completed the parasite larva leaves the host and moves a centimeter or two away where it spends 1 day as a prepupa before entering the pupal stage which lasts 6 days. Thus in a total of 11 days (1 as an egg, 3 as a larva, 1 as prepupa, and 6 as a pupa) the developmental period of this parasite is completed and the adult emerges after cutting a circular hole through the surface of the mine with its mandibles.

The most noteworthy feature of the biology of *S. begini* is its manner of pupation. When the mines of the parasitized hosts are opened for examination the pupa of *Solenotus* is found to be lying between two straight rows of symmetrically arranged pillars extending vertically from the floor of the mine to its ceiling (fig. 1). The pillars normally occur in four pairs although there are occasional sets in which a pillar may be either lacking, partially formed, or out of position. It would appear that these pillars support the ceiling of the mine and thereby prevent its collapse on to the otherwise unprotected and naked exarate pupa.

An almost identical habit was described by Howard (1882, 1891) in *Pleurotropis singularis* (Howard), a parasite of the lepidopterous leaf-miner, *Cameraria hamadryadella* (Clem.). In Howard's species there were 12 to 15 pillars arranged in an ellipse around the pupa. He found the distances between the pillars to be uniform and the pillars themselves to be very constant in size with a length of about 0.35 mm. Howard believed that the pillars were formed by regurgitated material, but in *Solenotus begini* it has been observed that they are formed entirely of meconium. This disposal of meconium to serve in an adaptive, protective manner is a remarkable example of the economy of parasitism.

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Notes on Parasites of Oriental Fruit Moth Cocoons[1]

M. H. BRUNSON, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

Many records of parasites of oriental fruit moth (*Grapholitha molesta* Busck) cocoons are based on small numbers of individuals. Information on the species of minor importance is therefore limited. In 1956 the author had the opportunity to obtain information on three species that have been reported infrequently. They were *Eupelmus limneriae* How., *E. cyaniceps cyaniceps* Ashm., and *Tritneptis hemerocampae* Grlt. They were obtained from laboratory-reared cocoons exposed in successive lots for 7 days on the trunk and large limbs of peach trees between May 25 and August 15. The cocoon remains were examined to determine the immediate host of each parasite reared. All species were reared as primary parasites.

In 8 years between 1931 and 1945 members of the Moorestown, N. J., Laboratory exposed thousands of cocoons to parasitization by trapping mature larvae on peach trees under burlap bands and in corrugated cardboard strips, and by pinning insectary-reared cocoons to trees. In the course of these studies records were obtained from eight States. Only two specimens of *E. limneriae* were reared, one from Beverly, N. J., in 1932 and the other from Parry, N. J., in 1934, and both were primary parasites. In 1956, however, the species was obtained in considerable numbers from orchards near Delango, Bridgeboro, Riverton, and Marlton, N. J., each month from May to August. Five per cent of 478 parasites from these localities reared during the year were of this species. Parasitism by all species averaged 18%.

E. cyaniceps cyaniceps had not been reared from the oriental fruit moth at Moorestown prior to 1956. One specimen was obtained from Delanco, N. J. (July 4–11), and one from Bridgeboro, N. J. (July 18–25). *T. hemerocampae* was obtained from one cocoon from Marlton, N. J. (June 29–July 6).

Determinations were made by B. D. Burks, of the Entomology Research Division, U. S. Department of Agriculture, Washington, D.C.

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How Much Can the Khapra Beetle Spread in the U.S.A.?[1]

R. W. HOWE[2] and D. L. LINDGREN[3]

In a paper given at the Tenth International Congress of Entomology at Montreal, Quebec, Canada, in August, 1956, the senior author described a method whereby the distribution and economic importance of stored-product insects could be predicted from laboratory observations. Briefly, simple life history data such as the developmental period, sex ratio, oviposition rate and mortality at various stages are found for a species at constant conditions and from these data a theoretical rate of increase of

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[2] Pest Infestation Laboratory, Slough, Bucks, England.
[3] Dept. of Entomology, Univ. of California Citrus Expt. Station, Riverside.

the species at these constant conditions is calculated. Comparison of the conditions in the environment under consideration with rates of increase at the appropriate constant conditions may be used as the basis of judgement of the local importance of the species.

Howe illustrated his paper by reference to the khapra beetle, *Trogoderma granarium* Everts, but his conclusions for the United States of America were not very precise, owing to the inadequacy of his climatic data for this country. In view of the widespread search for this species in the U.S.A. it seemed worth examining the extensive summary tables compiled by the U. S. Weather Bureau in order to determine which areas are most liable to invasion by the khapra beetle.

The method used to estimate the suitability of an area for the khapra beetle was to count the number of months for which the average temperature was 20° C. (68° F.) or higher. The lower developmental minimum for this insect is in fact about 21° C. (70° F.). It is considered that this beetle is not likely to multiply sufficiently to be a problem unless the average temperature exceeds 20° C. for at least 4 months. This is so over most of the southern half of the U.S.A. If an average of 20° C. is exceeded for 6 months or more, the khapra beetle is potentially a very serious pest, and these conditions are experienced in California, Arizona, and along the Gulf Coast from Texas to Florida. This method of delineating the khapra beetle area was adopted because it enabled a quick perusal of a mass of figures and is unlikely to underestimate the area liable to infestation. In practice, at temperatures above 27° C. (80° F.), multiplication is much more rapid than at lower temperatures. For instance, this insect would increase as much in 3 months each averaging 30° C. (86° F.) as in 12 months each averaging about 24° C. (75° F.). If a monthly average of 20° C. is reached for only 3 months, however, the average must reach at least 30° C. during 1 month, if the khapra beetle is to increase at all.

The picture as determined by temperature has to be modified by considering relative humidity, for at humidities over about 50% the khapra beetle does not compete successfully with the rice weevil (*Sitophilus oryza* (L.)), or lesser grain borer (*Rhyzopertha dominica* (F.)). Consequently the relative humidity during the hot months was considered. This was done very inadequately using the information given in the U. S. Weather Bureau National Summary, but these figures quite clearly exclude from the khapra beetle area all of the U.S.A. east of Kansas, Oklahoma, and Texas.

On climatic grounds, therefore, the only states needing to fear rapid multiplication of the khapra beetle over a wide area are California, Arizona, and Texas (fig. 1). In these areas the theoretical annual increase of this species varies between 100 and 40,000. Such multiplication cannot arise in practice, of course, but they illustrate the pressure of the problem. Nevada, New Mexico, Oklahoma, and Kansas have areas in which the species could also increase considerably. The potential annual increase in these areas having average temperatures over 20° C. for 4 months varies from only 1.5 to nearly 200, depending on the temperatures reached during the hottest months.

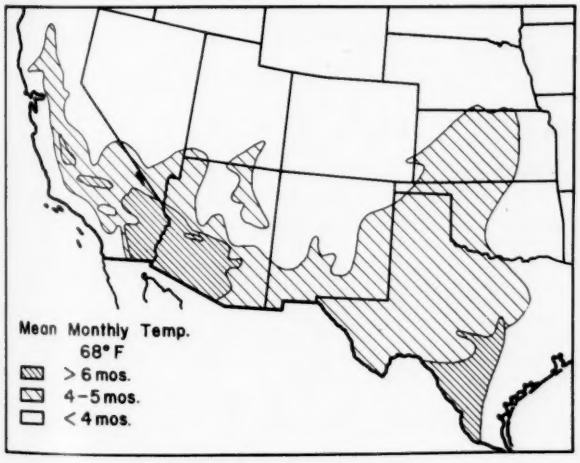

FIG. 1.—Climatic areas potentially favorable for khapra beetle development.

Prediction of the distribution of storage insects on a climatic basis must break down where storage environments are independent of temperature, particularly when buildings are artificially heated or when insects cause the heating of commodities. Thus, the khapra beetle is a pest in maltings in the United Kingdom although the average temperature never reaches 20° C., but it does so only because malt is very dry and is frequently stored at 25° to 40° C. Again in South Africa which, like much of the U.S.A., is only marginally suitable for this insect, the beetle has invaded grain storage probably taking advantage of heating induced by *Sitophilus* spp. In the northern and eastern U.S.A., therefore, the search for the khapra beetle should be maintained in heated premises and in any heating commodities which may be present. The most useful tools for the inspectors may be thermocouples for detecting heating, together with pieces of corrugated paper or some other material which can be placed in warehouses and grain bins as traps providing crevices for wandering larvae. This kind of trap has been used in Nigeria to follow changes of population intensity. In the areas too hot and dry for pests other than the khapra beetle, heating may be a fairly certain indicator of its presence. New infestations are likely to appear for several years, for this insect is not easily killed by cold or starvation, and it is difficult to find in small numbers. This latter point makes the khapra beetle an awkward inspection problem for port quarantine inspectors, especially those of California and Texas. Fortunately, this insect usually increases considerably in transit during a sea voyage. It is now present in all of Asia from India westward, and in most of Africa and is liable to appear in commodities from these places. It is not known from Australasia or South America and its distribution is very restricted in Europe.

New Distribution Record and Economic Importance of *Menacanthus cornutus* (Schömmer) on Georgia Broilers[1]

W. MALCOLM REID and ROBERT L. LINKFIELD,[2] *Poultry Department, University of Georgia, Athens*

During the course of a systematic survey of the incidence of parasitism of Georgia broilers a heavy infestation of *Menacanthus cornutus* (Schömmer) was discovered. This is the first time this species has been reported from Georgia. *M. cornutus* was first reported as occurring in the United States by Emerson (1956), who found it in abundance on farm flocks in Oklahoma. He also reported material in the United States National Museum collected from Auburn, Alabama. Since this species superficially resembles *M. pallidulus* (Newmann) and *M. stramineus* (Nitz), it seems probable that it has been frequently misidentified as the chicken body louse.

On August 24, 1956, a call reporting a heavy infestation of lice was received from the dressing plant in response to the parasite survey which was being conducted. Processed birds were downgraded as a result of reddish markings occurring on the shoulder areas. Hundreds of lice were present on each of the 3,500 birds in this flock. Plant workers were distressed by the lice crawling upon

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[2] The authors wish to acknowledge with thanks the assistance of Phyllis T. Johnson of the Entomology Research Division of the U. S. Department of Agriculture in identification of these specimens.

their persons. The service man in charge of the flock attributed poor feed conversion and poor growth to this infestation. Two weeks earlier some of the plant personnel had noted a similar but lighter infestation in another group of 3,500 birds which had been grown out in an adjacent broiler house belonging to the same grower.

A trip to the farm was made to find out more about the history of the infestation. A few culls still remaining in these two broiler houses harbored hundreds of lice. One of the culls had a deep wound an inch and a half in diameter in which masses of specimens were feeding. Blood was clearly visible in the intestinal tracts of lice taken from this area. A number of adult hens observed ranging in the yard had access to an area under the feed room of the two broiler houses from which the two sets of infested birds had been taken. Although it was not possible to examine the hens, it was assumed they had brought in the infestation. Under this assumption, the serious problem which developed in these two flocks emphasizes the desirability of keeping adult birds away from young stock. This management practice is also strongly recommended by many poultry authorities for control of many poultry diseases.

In the survey samples were taken from two or more flocks in each consecutive month from October 1955 to January 1957, representing 440,974 chickens in 100 farm flocks examined at market age in the processing plant. One thousand birds were individually examined by selecting at random 10 birds from each of the 100 flocks. Ten of the louse-infested birds were included in this survey, but would have been missed in the random flock selection had this particular group of infested birds not been called to our attention. All of the other 990 individually examined broilers were found entirely negative for ectoparasites, thus giving a 1% infestation for the survey. A more realistic estimate of economic incidence of parasitism might be obtained in another way. Plant personnel were aware of the search for ectoparasites but reported none in the broilers processed during this period except in the two previously mentioned flocks. By dividing the 7,000 birds in these two infested flocks by the 20,413,583 broilers processed in this period at the plant a 0.034% infestation is recorded. This method of calculating incidence, however, depends upon the casual observations of plant personnel who made no careful search rather than the more thorough individual bird examination as carried out on the 1,000 bird sample. Since birds are marketed at 8 to 10 weeks of age, it appears that this fast turnover under commercial broiler conditions does not permit serious ectoparasite populations to build up except under certain specialized conditions.

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IMPORTANT NOTICE TO AUTHORS

In accordance with action taken by the Editorial Board, each manuscript submitted for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY after September 1, 1957, must be accompanied by an abstract prepared in accordance with the requirements of Biological Abstracts, and typewritten (double spaced) *on a separate sheet* (or sheets) of paper. The abstract will replace the usual author's summary. A more detailed announcement of this requirement appears on page 1 in the Bulletin of the Entomological Society of America Vol. 3, June, 1957.

THOMAS SAY FOUNDATION, VOLUME II

A supply of Volume II, Plecoptera or Stoneflies of North America, by J. G. Needham and P. W. Claassen has recently been located. All five volumes that have been published by the Foundation are now available. Copies of Volume II sell for $5.00 postpaid in North America and U. S. Possessions, and for $5.50 elsewhere.

BOOK REVIEW

ANNUAL REVIEW OF ENTOMOLOGY, VOLUME 2, 1957. Edited by E. A. Steinhaus, *et al.* 8vo., cloth, 407 pp., illus., Annual Reviews, Inc., Stanford, California, in cooperation with Entomological Society of America. 1957. $7.00.

Entomologists and related scientists everywhere will be pleased to know that Volume 2 of the Annual Review of Entomology is now available. Those who have had the opportunity to study Volume 1 know what a valuable asset it is not only to the professional entomologist but to related scientists and others interested in entomology.

Volume 2 is written in much the same style as Volume 1 but an entirely different category of topics is introduced. Twenty-one were discussed in Volume 1 and 20 in Volume 2. The average length of each subject is approximately 19 pages including literature citations by authors and publication source. As in Volume 1 titles of the literature citations are omitted. This is of course unfortunate but since approximately 15 per cent of the total space is occupied by these reference citations, it would have been unwieldy to have included titles. Too, the purpose of the articles is not to present a literature review but to bring the reader up-to-date on the subject by making pertinent references to authorities in the field, which has been done admirably.

The committee is to be congratulated on the diversity of subjects selected and particularly on the choice of authors to present the subject matter. Nowhere else would it be possible to find so authoritative a discussion of 41 topics covering so broad a field of entomological interests as in Volumes 1 and 2 of these reviews. In order that those interested may be familiar with the contents, they are as follows by authors and volume:

Volume 1

The Physiology and Biochemistry of Diapause—A. D. Lees
Insect Nutrition—H. Lipke and G. Fraenkel
The "Language" and Orientation of the Honey Bee—K. von Frisch and M. Lindauer.
The Stability of Scientific Names—R. L. Usinger
Some Aspects of Geographic Variation in Insects—T. H. Hubbell
Arthropod Resistance to Chemicals—W. M. Hoskins and H. T. Gordon
The Mode of Action of Insecticides—C. W. Kearns
The Chemistry of Insecticides—H. Martin
Persisting Insecticide Residues in Plant Materials—F. A. Gunther and R. C. Blinn
Repellents—V. G. Dethier
Soil Insects and Their Control—J. H. Lilly
Stored Product Entomology—E. A. Parkin
Apparatus for Application of Insecticides—J. L. Brann, Jr.
Ecology of Forest Insects—S. A. Graham
Some Recent Advances in Apicultural Research—C. G. Butler
Insect Transmission of Plant Viruses—F. F. Smith and P. Brierley
Nonbiting Flies and Disease—D. R. Lindsay and H. I. Scudder
Veterinary and Medical Acarology—H. S. Fuller
Modern Quarantine Problems—A. F. Camp
The Fundamental Theory of Natural and Biological Control—W. R. Thompson
Effect of Pesticides on Balance of Arthropod Populations—W. E. Ripper

Volume 2

Digestion in Insects—D. F. Waterhouse
Some Aspects of Intermediary Metabolism of Carbohydrates in Insects—M. Rockstein
The Physiology of Insect Cuticle—V. B. Wigglesworth
The Comparative Morphology of the Insect Head—E. M. DuPorte
Cytogenetics and Systematic Entomology—M. J. D. White.
The Taxonomic Significance of the Characters of Immature Insects—F. I. van Emden
Caste Determination in Social Insects—M. V. Brian
Dynamics of Insect Populations—M. E. Solomon
The Synoptic Approach to Studies of Insects and Climate—W. G. Wellington
Insect Migration—C. B. Williams
Recent Advances in Veterinary Entomology—A. W. Lindquist and E. F. Knipling
Transmission of Disease Agents by Phlebotomine Sand Flies—S. Adler and O. Theodor
Genetics of Insect Resistance to Chemicals—J. F. Crow
The Mode of Action of Insecticides Exclusive of Organic Phosphorus Compounds—P. A. Dahm
Chemistry and Mode of Action of Organophosphorus Insecticides—E. Y. Spencer and R. D. O'Brien
The Behaviour of Systemic Insecticides Applied to Plants—S. H. Bennett
Aerial Application of Insecticides—F. E. Weick and G. A. Roth
Cotton Insects and Their Control in the United States—J. C. Gaines
Insecticidal Control of the Spread of Plant Viruses—L. Broadbent
Pollination of Alfalfa and Red Clover—G. E. Bohart

In general the discussions of the various subjects are thoroughly readable. The authors have attempted to avoid highly technical terminology and even though some of them, particularly those dealing with the chemistry and mode of action of insecticides, are somewhat "over the head" of the layman, they are well within the grasp of the average professional. As would be expected, there is considerable variation in the manner in which the different authors have presented their subject matter. In general, however, it is well organized and in all cases it is well fortified with citations to the literature.

This Annual Review series will become more valuable with each volume. Already the 41 articles contained in the first two volumes provide the reader with a wealth of timely entomological information in condensed form. Volume 3, in preparation, will contain an additional 20 articles of timely interest. These will be useful as ready reference sources, as working tools for the researcher, as supplementary teaching material at both the graduate and upper undergraduate levels, and as authoritative reading matter for those interested in the latest developments in entomology.

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- X grape leafhopper
- X grape leaf folder
- X green June beetle
- X imported cabbageworm
- X Japanese beetle
- X Mexican bean beetle
- X oriental fruit moth
- X pink bollworm
- X plum curculio
- X potato leafhopper
- X red-banded leaf roller
- X rosy apple aphid
- X southern armyworm
- X spinach leaf miner
- X squash bug
- X tobacco budworm
- X tobacco hornworm
- X yellow-striped armyworm
- X and others

CRAG BRAND AGRICULTURAL CHEMICALS

CRAG Agricultural Chemicals

CARBIDE AND CARBON CHEMICALS COMPANY

A Division of Union Carbide and Carbon Corporation

30 East 42nd Street UCC New York 17, New York

"Crag" and "Sevin" are trade-marks of Union Carbide and Carbon Corporation

LEADING GROWERS OF MANY CROPS DEPEND ON TOXAPHENE FOR SEASON-LONG INSECT CONTROL. FOR EXAMPLE, TOXAPHENE IS OFFICIALLY RECOMMENDED FOR CONTROL OF MORE COTTON INSECTS THAN ANY OTHER INSECTICIDE.

Agricultural Chemicals Division, Naval Stores Department
HERCULES POWDER COMPANY
INCORPORATED
991 Market Street, Wilmington 99, Del.

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